CO_2 CAPTURE, NUTRIENTS UPTAKE AND LIPIDS CONTENT OF MICROALGAE SPECIES CULTURING IN WASTEWATER MEDIA

BY

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DEDICATION

To my parents and my family

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Firstly, I am thankful to Allah almighty for his blessing and for giving me the confidence and the patient to complete this work. I would like further to acknowledge King Fahd University of Petroleum & Minerals (KFUPM) for providing a full scholarship to purse the M.Sc. program. Also KACST for funding the thesis project. Special thanks to all faculty members of Chemical Engineering Department for their support during this program.

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ABSTRACT

Full Name : Saad Aldin Mohamed Ali

Thesis Title : CO_2 Capture, Nutrients Uptake and Lipids Content of Microalgae Species Culturing in Wastewater Media

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This research deals with an experimental investigation of integrated CO_2 (from flue gas) conversion, wastewater treatment and biofuel production by growing microalgae. In this regard, two microalgae species (*Nannochloropsis oculata* and *Chlorella vulgaris*) have been considered. The rate of CO_2 bio-conversion is determined by measuring the total organic carbon content of microalgae species. The nutrients uptake during cultivation period, lipids extraction and biomass thermal conversion are also studied. The results show that under phototrophic condition microalgae cells consume higher amount of CO_2 than that of mixotrophic condition. The rate of CO_2 bio-fixation under phototrophic mode are 1.64 and 1.96 gCO_2/g biomass for *Nannochloropsis oculata* and *Chlorella vulgaris,* respectively. The nitrogen compounds uptake from the municipal wastewater depends on the initial concentrations and N/P ratio. The phosphorus compounds can be removed completely within short period. In lipids extraction, the disruption of microalgae cells significantly enhances the lipids amount. The cells disruption by sonication gives almost three time higher crude lipids than the amount without sonication. The thermal analysis of the produced biomass shows that the microalgaes are thermally stable up to 200°C. Following that, the microalgal biomass is sharply decomposed within 600°C. The thermal oxidation kinetics modeling is conducted using TGA data at various heating rates. The estimated apparent activation energies are found to be 152 kJ/mol and 214 kJ/mol for *Nannochloropsis oculata* and *Chlorella vulgaris*, respectively.

ملخص الرسالة

الاسم الكامل: سعد الدين محمد علي

عنوان الرسالة: احتجاز ثاني أكسيد الكربون، امتصاص المغذيات ومحتوى اللبيدات لأنواع الطحالب الدقيقة المزروعة في مياه صرف صحي

التخصص: الهندسة الكيميائية

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إن هذا البحث يختص بالدراسة المبنية على التجارب للتحقق من إمكانية دمج تحويل ثاني أكسيد الكربون (من غاز المداخن) مع معالجة مياه الصرف الصحي وإنتاج الوقود الحيوي بواسطة زراعة الطحالب الدقيقة. لهذا، تم اعتبار نوعين من الطحالب الدقيقة (نانوكلوربسس اوكيولاتا و كلوريلا فالجاريس). لقد تم حساب معدل التحويل البيولوجي لثاني أكسيد الكربون بقياس المحتوى الكلي للكربون العضوي لنوعي الطحالب الدقيقة. كذللك تم دراسة امتصاص المغذيات خلال فترة الزراعة، استخلاص اللبيدات، والتحويل الحراري للكتلة الحيوية الناتجة. النتائج المتحصل عليها تشير على ان في حالة الزراعة في بيئة ضوئية التغذي فان خلايا الطحالب الدقيقة تستهلك معدلات عالية من ثاني أكسيد الكربون مقارنة بالزراعة في بيئة مختلطة التغذي. حيث بلغ معدل التحويل البيولوجي لثاني اكسيد عند الزراعة في بيئة ضوئية التغذي 1,64 و 1,96 جم ثاني أكسيد الكربون\جم كتلة حيوية لنوعي الطحالب الدقيقة نانوكلوربسس اوكيولاتا وكلوريلا فالجاريس على التوالي. ان امتصاص وازالة مركبات النيتروجين من مياه الصرف الصحي تعتمد على تراكيزها الابتدائية وعلى نسبة النتروجين الى الفسفور. كما يمكن إزالة مركبات الفسفور بشكل تام في وقت قصير. اما بخصوص استخلاص اللبيدات، فان احداث تشوهات في خلايا الطحالب الدقيقة يساعد فعليا في تعزيز كمية اللبيدات المستخلصة. حيث وجد ان استخدام الموجات الصوتية لتشويه خلايا الطحالب يضاعف كمية اللبيدات الخام اكثر من ثلاث مرات مقارنة بعدم استخدام الموجات الصوتية. ان نتائج التحليل الحراري للكتلة الحيوية الناتجة أظهرت ان الطحالب الدقيقة مستقرة حرارياً في درجة حرارة اقل من 200 درجة مئوية. اما بعد ذلك، فان كتلة الطحالب الحيوية تتحلل حراريا بشكل كلي عند الوصول لدرجة حرارة 600 درجة مئوية. تم تمثيل حركيات الأكسدة الحرارية با ستخدام معطيات متحصل عليها من محلل حراري وزني، وباستخدام معدلات تسخين مختلفة. لقد وجدت طاقة التحفيز الظاهرة حوالي 152 كج\مول و 214 كج\مول لنانوكلوربسس اوكيولاتا وكلوريلا فالجاريس على التوالي.

CHAPTER 1

INTRODUCTION

1.1. Overview

1.1.1. Biological CO_2 Capture

Global warming has become a current issue of concern in the world today. The main sources of global climate change are the increasing concentrations of greenhouse gases that come primarily from human activities and power plant operation. [1]. Scientists are seeking to find solutions to global warming through the mitigation of the greenhouse gases. Carbon dioxide (CO_2) is a well-known greenhouse gas that is released from natural sources. Other than the CO_2 released from nature, the CO_2 produced as a result of industrialization and human activities, can greatly increase its concentration in the atmosphere [2]. The French National Center for Scientific Research (CNRS), has reported that the amount of carbon dioxide emitted to the atmosphere has increased dramatically during the last 100 years [3]. This rise in carbon dioxide emissions, is mainly due to the combustion of fossil fuels, which have been on the increase due to the growth in world population [4].

In particular, fossil fuel combustion as a source of energy in power plants is a major source of the CO_2 released to the atmosphere [5]. The reduction of the CO_2

generated from fossil fuel combustion in power plants can be achieved by improvements in the power plant generating efficiency, the use of other clean sources of fuels, and by CO_2 capture and storage technologies [1].

Although, there are different CO_2 capture approaches, however the biological CO_2 capture method is an attractive approach. Carbon dioxide can be converted by photosynthesis to organic matter by utilizing the sunlight as a source of energy. Generally, terrestrial plants can take CO_2 and produce organic matter through photosynthesis. In addition, algae can convert CO_2 into organic compounds more efficiently and this when compared with other terrestrial plants. Particularly, microalgae are preferable to macroalgae because they are easy to be cultivated using mass culture methods [1].

1.1.2. Wastewater Treatment Using Microalgae

The utilization of microalgae to remove nutrients (especially nitrogen and phosphorus compounds) from wastewater is such a green technology that reduces or replaces the use of chemicals in the treatment stages. Thus, the benefits of this technology are to allow both CO_2 capturing through photosynthesis and removing of the nutrients from wastewater [6], [7].

Micro contaminants in wastewater effluents can cause serious problems if they infiltrate the drinking surface water treatment process. Micro pollutants present in wastewater discharges are very difficult to be removed and require special treatments. Even though advanced oxidation processes are usually conducted

quite effectively and often used in the drinking water treatment , these methods do not guarantee the complete removal of micro contaminants [8].

For these reasons, microalgae can play an important role in removing micro contaminants from wastewater. Microalgae can also reduce the amount of nutrients (carbon, nitrogen and phosphorus), generating a considerable amount of oxygen which is available for the decomposition of organic matter by bacteria. Microalgae can also mitigate wastewater bad odour issues [9]. Specifically, municipal wastewater provides a good option for microalgal biomass production. This is because municipal wastewater effluents are produced in large amounts and are rich in nutrients.

1.1.3. Biodiesel Production from Microalgae

Over the past few years, a significant number of researchers have been interested in the production of biofuel from microalgae. However, this avenue it is now being considered very carefully , owing to the increase in the selling price of fossil fuels together with the rising concerns about the environmental impact of fossil fuel burning emissions, and very specially the one of global warming [10].

Many microalgae have high oil content, thus, they can be converted via chemical reaction into biodiesel. However, the oil content of some microalgae species can reach 80% on a dry weight basis [11], [12]. Biodiesel production from microalgae is considered as one of the most effective strategies for biofuels generation, and it is also seen as an appealing alternative to meet the current demand for alternative fuels [13]. Moreover, replacing fossil fuels with biodiesel or other biofuel products

from the conversion of microalgal biomass can contribute to the reduction of CO_2 emissions and hence minimize the combustion of the fossil fuels [14].

1.1.4. Integrated Approach

Biological CO_2 fixation using microalgae could be combined with other processes like wastewater treatment. This would be advantageous to offer more economical feasibility and environmentally sustainability. However, researchers should consider the advantage of placing waste water treatment plant in the proximity of power plants, where there may be significant CO_2 available from the flue gases of fossil fuel combustion.

When microalgae is cultured in wastewater rich of nutrients, this provides a source of food for microalgae to grow. The resulting biomass can be used as a feedstock for biofuel production. Therefore, the combination of CO_2 capture, wastewater treatment and biofuel production provides an attractive strategy to current CO_2 capture methods [6]. Hence, this method captures flue gases, which are a rich source of CO_2, minimizing the lifecycle energy requirements of biomass. Furthermore, the resulting biomass can be further processed to produce biofuel and other by-products[6], [7].

After microalgal biomass is harvested from the wastewater culture, biofuels can be produced through different conversion methods. Some examples of this are (i) the production of bioethanol by fermentation, (ii) the production of biodiesel through the transesterification of lipids, or (iii) the generation of fuel gas by biomass gasification[15]. As well, microalgal biomass conversion methods can be classified

as thermochemical, biochemical and chemical based reactions technologies, as illustrated in Figure 1-1. Figure 1-1 describes the combined CO_2 capture, wastewater treatment and biodiesel production using microalgae.

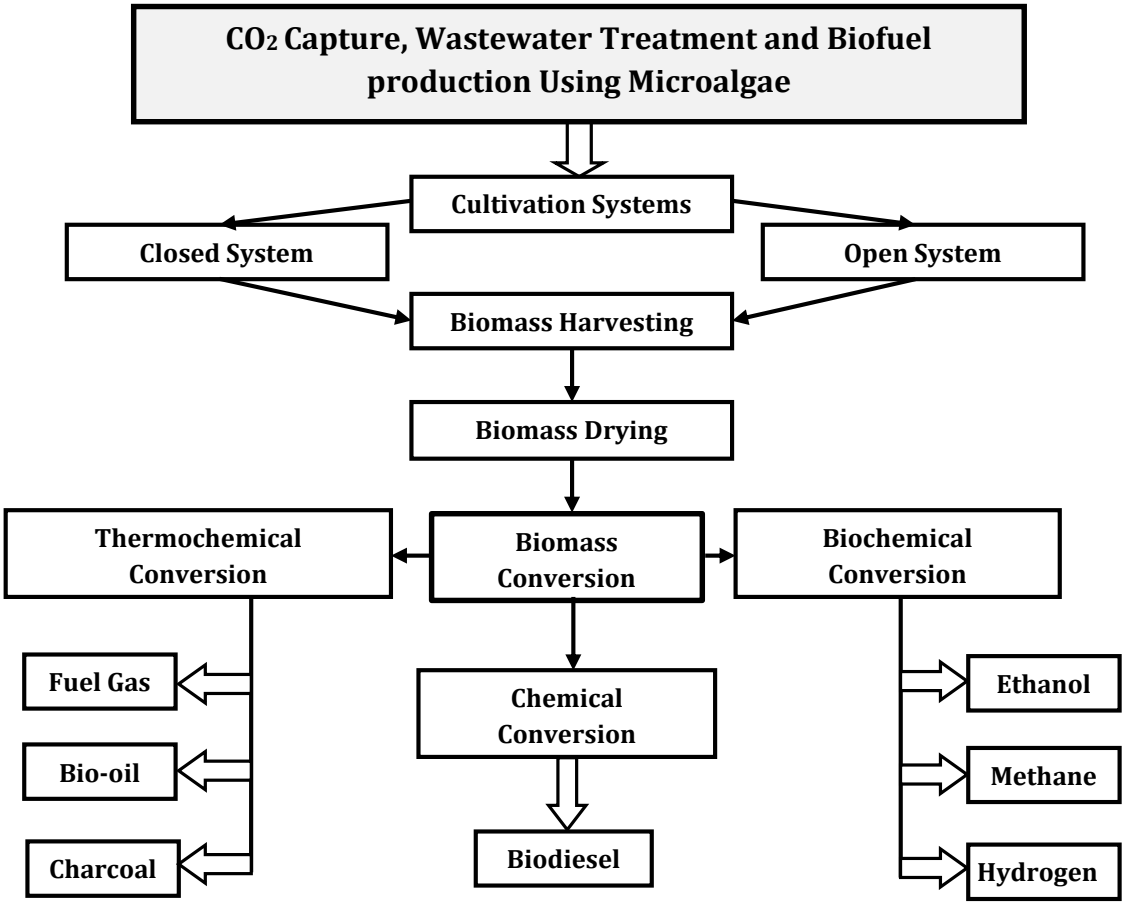


Figure 1-1 Integrated CO_2 Capture, Wastewater Treatment and Biofuel Production Using Microalgae

Typically, microalgae are cultivated in the wastewater using either the open system or the closed system. The growth media is controlled to achieve the removal of nutrients from wastewater with maximization of the biomass produced with targeted quantity. After that, the biomass is harvested, and analyzed. Biomass

thorough analysis is critical in order to find the best approach in which to convert biomass into the desired end products. In most cases, it is preferred that microalgal biomass be converted into biodiesel as mentioned earlier.

Nevertheless, the large scale microalgae production and harvesting, offer still challenges towards the application of an integrated CO_2 capture, wastewater treatment and biofuel production. This should be done in a way that provides the opportunity to produce valuable biofuels and other non-fuel bioproducts. Thus, further investigation into the downstream process for the production of biofuels and other non-fuel bioproducts is necessary. In addition, there are important challenges related to the large-scale production of microalgae such as the supplying and recycling of nutrients, gas transfer, and photosynthetic efficiency [16]. Moreover, the extraction of lipids from microalgal biomass is an important factor for the scale-up of microalgal biodiesel production and for the understanding of how this may affect its economic viability [17].

1.2. Structure of Thesis

- **Chapter 1:** The introductory chapter gives the general trend of the thesis. In particular, the utilization of microalgae for different approaches: i) CO_2 capture, ii) wastewater treatment iii) and also for biofuel production.
- **Chapter 2:** This chapter includes a detailed literature review in the area of microalgae culturing. Researches shortcoming was highlighted clearly in order to declare the thesis objectives
- **Chapter 3:** The overall and specific thesis objectives were listed
- **Chapter 4:** This section involves demonstration of the method for culturing microalgae, and the composition of culture media being used. Besides, presenting a full description of the analytical methods used in these investigations.
- **Chapter 5:** Investigation about CO_2 fixation by culturing microalgae under phototrophic and mixotrophic condition.
- **Chapter 6:** Study of phosphorus and nitrogen compounds uptake and removal from wastewater media by using microalgae culturing.
- **Chapter 7:** Analysis of the thermal conversion of microalgal biomass under oxidative atmosphere.
- **Chapter 8:** Investigation about extraction of lipids from biomass by organic solvent. In addition, the effects of the cells disruption on the extracted crude lipids content.
- **Chapter 8:** Conclusion and recommendations.

CHAPTER 2

LITERATURE REVIEW

2.1. CO_2 Capture Using Microalgae

In general, several CO_2 mitigation methods have been developed. These methods can be grouped into main two categories: (i) chemical reaction-based methods and (ii) biological methods [6]. The chemical reaction methods include cyclic carbonation and de-carbonation reactions. These methods involve the reaction of CO_2 with a metal oxide to produce a metal carbonate [18]. Physical-chemical strategies for CO_2 capture from fossil fuel burning power plants are still considered expensive processes [5].

On the other hand, the biological CO_2 capture processes have been explored to find solutions for the global warming problem [19]. According to Chisti [12] microalgae biomass contain 50 % carbon, however around 1.83 kg of CO_2 can be captured by 1 kg of biomass produced.

Most of the studies in the area of CO_2 bio-fixation using microalgae were based on the measurement of CO_2 concentration at the inlet and outlet of the culturing reactor. However, this procedure may not be fully accurate since we cannot guarantee that the reduction in CO_2 concentration is a result of CO_2 consumption by the microalgae cells. Meanwhile, carbon content analysis gives exactly the amount of CO_2 consumed by cells.

2.2. Municipal Wastewater Treatment

2.2.1. Background

Wastewater treatment is performed for pollution control whether in an industrial application or for a municipal purpose. In the past, wastewater was released directly to the discharge to rivers, lakes and oceans. . Given that the population of the earth is rising, ways of treating this wastewater have to be established . In addition, the rapid growth of industry, results in significant amounts of wastewater. If not treated well, this could cause serious pollution problems. Water pollution control is a global concern and the conservation of ecosystems and the environment are a must [20].

Municipal wastewater is treated using chemical, physical, and biological methods to remove the undesirable compounds that cause environmental problems. The biological treatment using microorganisms, namely bacteria, is used to decompose the organic matter present in the wastewater effluents. In the aerobic process, a sufficient amount of oxygen is inserted into the system by different mechanisms. It is relatively expensive and requires a lot of effort to be conducted. However, it is interesting to note that microalgae produce oxygen by a photosynthetic reaction which can be used in the aerobic degradation process. However, microalgae are primarily used to remove the nutrients from the wastewater discharges [21].

2.2.2. Wastewater Nutrients

Nutrients are the main chemical elements and compounds existing in the environment, and are classified into macronutrients like carbon, nitrogen and

phosphorus compounds, and micronutrients such as trace metals and vitamins. The most frequently encountered nutrients are nitrogen and phosphorus compounds such as nitrate, nitrite, ammonia, organic nitrogen and phosphates. High levels of nutrients in the wastewater effluents cause eutrophication, which is the undesirable growth of plants and algal bloom in the discharge sinks like rivers, lakes and oceans. These plants consume a considerable amount of oxygen for their growth and that definitely reduces the dissolved oxygen content in the waters sources, placing the life of fishes and other microorganisms in danger. This phenomena is called hypoxia which refers to the depletion of oxygen in the water [22].

2.2.3. Basic Wastewater Treatment Processes

The main objective of the wastewater treatment processes is to accelerate the natural processes of water self-purification [20]. Physical, chemical and biological treatments are used with the intention of reducing pollutant concentration to lower specific values that meet the environmental regulations. The combination of these processes can be categorized as primary, secondary, and tertiary or advanced wastewater treatments. Thus, different treatment processes are needed in each stage to provide a specific level of treatment as illustrated in Figure 2-1 [23]. One should notice that there are sophisticated technologies that can be used, depending on the type of contaminants to be removed and the desired purification limit.

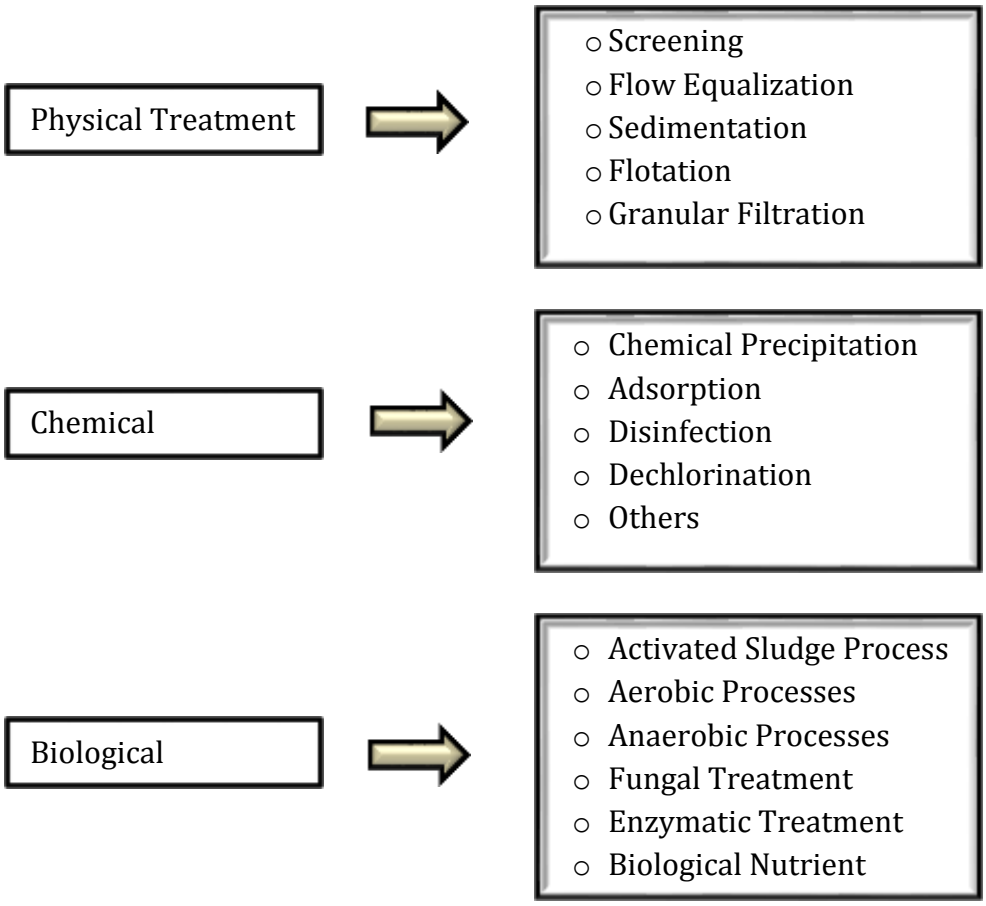


Figure 2-1 Wastewater Treatment Unit Operations- Modified from [23]

2.3. Biological Nutrients Removal Using Microalgae

Microalgae which refers to small algae, can be effectively utilized to remove the nutrients in the wastewater in the tertiary treatment phase. Domestic wastewater includes substantial amounts of nutrients available for algae growth [24]. Accordingly, microalgae is more efficient than other terrestrial plants for CO_2 capture [25]. Microalgae consumes nutrients to yield environmentally friendly constituents like lipids and carbohydrates, and also oxygen [26]. This is unlike traditional biological treatments, that use microorganisms to decompose organic

materials. These microorganisms produce CO_2 and activated sludge which is comprised of biological flocks. However, biofuel products could potentially be obtained from microalgae biomass [27].

Nowadays, much research is oriented toward studying the viability of microalgae use to remove nitrogen and phosphorous compounds from wastewater discharges [28]. In the early 1970's, research started to use algae for the treatment of secondary plant effluents. Researchers recommended to use the algal system rather than tertiary treatments, given its economic feasibility [26]. Wang et al. [26] studied the potential use of green algae *Chlorella sp*. to remove wastewater nutrients from the effluent of the primary treatment unit. The extent of total nitrogen and phosphorous removal were 68.5 % and 90.6% respectively, while the COD (Chemical Oxygen Demand) percentage removal was 56.5%. This confirmed that this type of algae was not very effective for the removal of organic matter from wastewater medium.

Wang and Lan [29] investigated the removal of nitrogen and phosphorous using a microalgae strain named *Neochloris oleoabundans* cultured in a synthetic secondary municipal wastewater effluent. Results showed that nitrogen compounds removal varied from 78% to 99% depending on the N/P ratio. The phosphorous compound was completely removed from the medium, since it was independent of the N/P ratio.

2.4. Microalgae Cultivation Systems

The selection of the microalgae cultivation system is the key to efficient nutrient removal and effective biomass production. Generally, the algae cultivation systems are classified as open systems or closed systems (photobioreactors) [30]. Hybrid systems, which are a combination of an open system, and a closed system, can be used to eliminate the disadvantages of both. This is required in order to achieve high biomass productivity with high nutrient removal.

2.4.1. Open Systems

Open ponds are widely used for microalgae culturing. These open ponds allow microalgae to uptake CO_2 from the air directly from the ambient atmosphere [6].

Types / Design

Different types and configurations of microalgae cultivation systems are used for microalgal biomass production and for water purification. The most commonly used systems for industrial algae cultivation and for research are the raceway pond, the circular pond tank, the shallow big pond and the closed pond, just to name a few. A proper selection of pond location is required to ensure light availability. Location is also relevant with respect to the type of algal species to be cultured [31], [32].

Advantages

Open systems can be constructed using different types of materials. From an economic standpoint, the open system is not very expensive. Moreover, it is easy

to clean with this being a good feature of this kind of system [13].Another advantage of an open system is the usage of sunlight which reduces the operation cost. Furthermore, it is easy to operate. [31], [33].

Disadvantages

Open cultivation systems are exposed to variations in weather conditions. This makes them difficult to control. Seasonal weather fluctuations may affect the light intensity and temperature. Considerable amounts of water will also possibly evaporate, resulting in a reduction of water being treated. Furthermore, the water level should be kept very low, to allow sunlight to reach all the microalgae being cultured [34]. Therefore, an open cultivation is recommended for regions that offer high solar radiation [35].

In an open system, other contaminants or other microorganisms can compete with the microalgae for their food. These competing microorganisms may limit algae growth. Thus, in open systems the microalgae may grow under unfavourable conditions, and as a result very few types of microalgae species are suitable for this type of open systems [32].

The availability of land area for microalgae culturing is another critical issue due to the large areas required for open cultivation. In addition, the CO_2 in the atmosphere is normally just around 0.03%. This shortage in CO_2 definitely limits the CO_2 mass transfer process required for photosynthesis [34].

2.4.2. Closed Systems (Photobioreactors)

In the closed systems, otherwise designated as photobioreactors (PBRs), growth conditions are well controlled. Additionally, single species cultivation can also take place [36]. Since photobioreactors solve many problems which are part of the open cultivation, researchers have recently focused on designing photobioreactors for large microalgal biomass production [35]. Generally, these reactors are designed to increase the light accessibility. They also allow perfect mixing, to permit the light to be within an optimum value for cell growth and to improve gas exchange [31].

Types / Design

Several classifications of photobioreactors are available in the literature, and can be grouped based on their geometry or depending on the material used for the reactor construction. Thus, the photobioreactors can be built as bags, tanks, and towers. Photobioreactors can be manufactured as plates or be tubular in shape. The materials of construction can be glass or plastic [37], [38].

Advantages

Closed systems offer effective and better light utilization which reflects positively on the biomass production. Due to the absence of other contaminant microorganisms, closed photobioreactors are capable of holding various kinds of microalgae species, and can be operated under different weather conditions [31]. Photobioreactors also reduce the CO_2 losses, since the gas flow rate and the mixing can be regulated without difficulty [34]. Additionally, temperature can be adjusted to a desired value by placing the reactor into a special room with a controlled

temperature. This is favorable given the difficulty of controlling cultivation temperature outdoors [39].

Furthermore, flue gases with a high level of CO_2 concentration, can be dosed into the microalgae culture, in a closed photobioreator system with all this contributing to the capture the CO_2 [6].

Disadvantages

Since there are different types of photobioreactors, they, thus, differ in their cost and in other operating requirements. It is therefore, difficult to compare them [40]. Closed system photobioreactors usually require high installation and operating costs. Hence, they are considered more expensive than open cultivation systems. There are also other drawbacks, like difficulties in reactor scale-up and the occurrence of biofouling [34]. Furthermore, there are some challenges related to cleaning procedures, and considerable efforts have been oriented to generate self-cleaning systems [40]. Chisti [12] postulated that some algae can grow and stick to the reactor walls. This lowers the light accessibility to the culture, and therefore, leads to a reduction in biomass productivity.

2.4.3. Hybrid Systems

Hybrid systems overcome the limitations of open systems and the high initial and operating cost associated with closed systems. Hybrid systems are cost-effective and can be used for large algae cultivation [13]. In this case, microalgae are first cultured in a photobioreactor, to achieve high density inoculants. Microalgae is then, moved to an open system, helping to reduce the holding time for optimum

biomass production [38]. Furthermore, the effects of contamination problems in open systems are considerably reduced. This is the case given that the desired microalgae species is cultured first in a closed system, and then transferred to an open system when a significant microalgae concentration is reached. Thus, when transferred to the open system, microalgae becomes immediately the dominant species competing very effectively with other microorganisms [41].

2.5. Microalgae Growth Parameters

For the combined wastewater and algal culturing, several factors must be considered. These factors are: i) the average residence time, ii) the nutrient supply degree of mixing and iii) the gas flow rate. Other criteria of importance are the measurement of nutrient removal characterized by BOD reduction, and the nitrogen and phosphorus chemical species reduction. However, the cultivation system has to be designed and operated to maximize the microalgae cell growth as well as the treatment of wastewater effluents [21].

Researchers are actively investigating the effects of growth conditions on the microalgae cell growth, the nutrient removal and the CO_2 capture. This is achieved by using different microalgae species and different types of reactors and configurations. For example, Yun et al. [19] studied the influence of phosphorous addition, CO_2 concentration and pH on the growth of *Chlorella vulgaris* cultured in the effluent of a steel-making plant. This researcher examined the CO_2 fixation and the ammonia removal in order to design an effective and economical reactor system.

There is a significant amount of research addressing the maximization of microalgae productivity. In this respect, a diversity of culture media have been used, ranging from fresh water to nutrient rich media like seawater, municipal and animal wastewater effluents. Experimental work has been conducted using several microalgae species with growth metabolisms. Generally, the adjustable parameters involved are: a) the concentration of macronutrients, b) the CO_2 concentration, c) the gas flow rate, d) the culture media temperature, e) the light intensity and f) the photosynthetic efficiency. The effects of each factor on the amount of biomass produced were already discussed in this review.

Figure 2-2 reports the various factors and conditions that affect the microalgae growth, providing a schematic representation of cell growth parameters. Later on in this review, the other factors that affect biomass productivity, such as lipids content and the nutrient composition will be considered. This is critical given that the maximization of lipids and free fatty acids is a major goal for large scale biofuel processes.

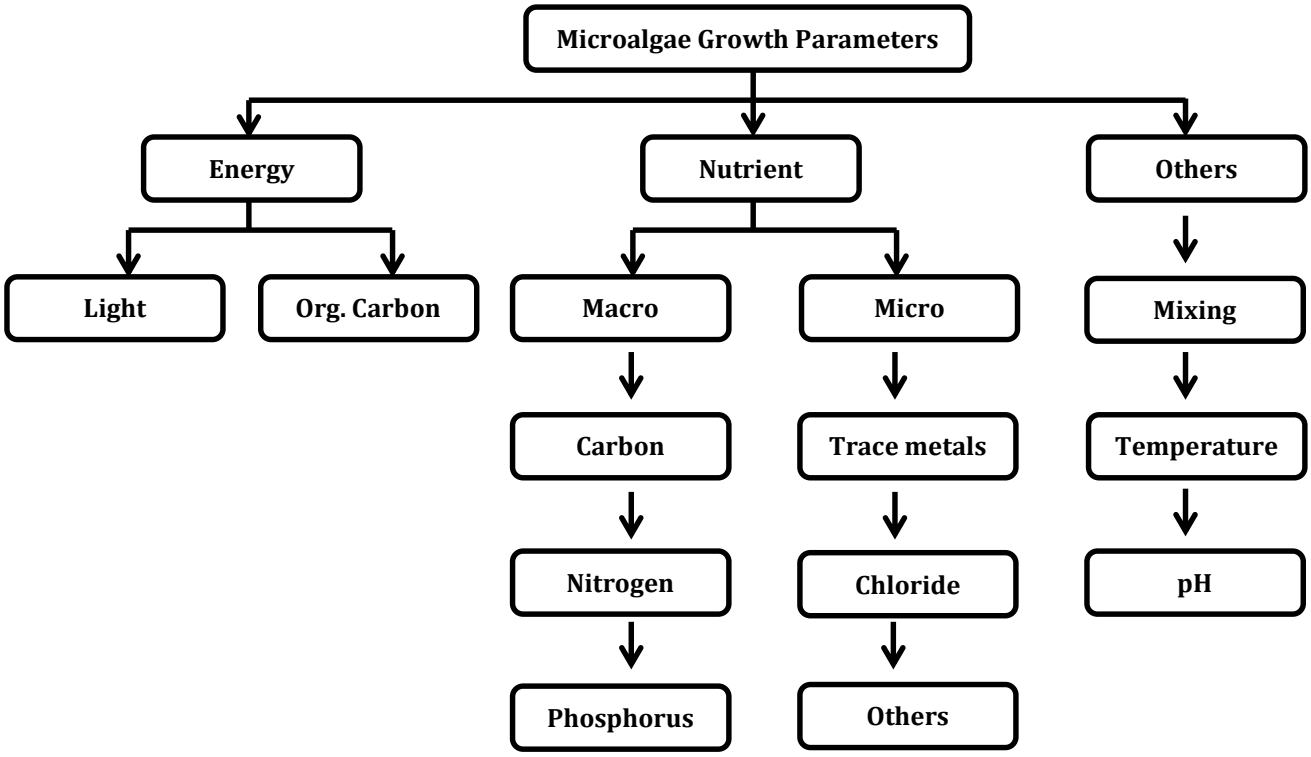


Figure 2-2 Schematic Diagram of Microalgae Growth Parameters Including Energy Source, Nutrient and Others Factors.

2.5.1. Cultivation Conditions

Microalgae can grow under different conditions depending on the source of energy and the carbon used. For instance, microalgae cells can grow under visible light and have inorganic carbon in the culture medium as the only carbon source. This is called photoautotrophic growth. Furthermore, organic carbon can be used as a food and energy source in heterotrophic growth. When both organic and inorganic carbon are employed as a food and energy source with and without visible light, this growth is designated as mixotrophic growth. Finally, photoheterotrophic growth is used to designate the microalgae culture where cells metabolize organic compounds as a carbon source in the presence of light [42], [43].

Photoautotrophic Culture

In photoautotrophic culture, cells utilize inorganic carbon like CO_2 as a carbon source in the presence of visible light. In this type of culture, the medium contamination is in most cases, less significant [44]. Photoautotrophic cultivation is used in both open and closed systems, since microalgae can utilize both CO_2 and light for cell growth. Photoautotrophic cultivation can produce polysaccharides, proteins, lipids and hydrocarbon compounds through photosynthesis. This is the case, as a result of microalgae having a high photosynthetic efficiency and growth rate compared to other plants [45].

Heterotrophic Culture

In the heterotrophic culture, microalgae use organic carbon matter both as an energy source and as the single carbon source. Hence, light is no longer required for cell growth. [46]. It is expected that the microalgae cultured under these conditions should be capable of growing in the dark and should have the ability to adapt quickly to a new culture media [47]. One should notice that under heterotrophic cultivation, the lipids productivity is much greater (about 20 times) than in the photoautotrophic cultivation. [44]. This increased lipids productivity may depend on the type of microalgae species, the culture medium composition, and other growth parameters.

Economically, heterotrophic cultivation is considered less expensive than photoautotrophic cultivation, since the light is no longer required for cells growth. In heterotrophic cultivation, both reactor design and scale-up are relatively straightforward. It is also anticipated that heterotrophic culture may be suitable

for the processing of large volumes of medium such is the case of wastewater effluents [48].

Mixotrophic Culture

In mixotrophic cultivation, microalgae can grow autotrophically or heterotrophically depending on the light availability and the concentration of carbon source compounds [34]. A mixotrophic metabolism can be classified depending on microalgae nutrition habits being, either of facultative and/or of obligatory types [49]. One should mention that for some microalgae species, the mixotrophic specific growth rate expressed in h^{-1} is almost the sum of both the photosynthetic (photoautotrophic) and the heterotrophic specific growth rates [50].

Photoheterotrophic Culture

Microalgae growing under photoheterotrophic conditions use visible light as an energy source only. Furthermore, in a photoheterotrophic metabolism, cells utilize organic carbon for growth. On the other hand, in mixotrophic cultivation, the energy source can be either light or organic carbon compounds. In addition, in mixotrophic growth both organic and inorganic carbon are used as the carbon source [42].

Photoheterotrophic microalgae cultivation can be used to produce hydrogen [51], [52]. One can also notice that there is, however, no mention in the technical literature regarding research developed using photoheterotrophic cultivation for lipids or biodiesel production.

Das et al. [53] study the two phase growth of *Nannochloropsis Sp*. under photoautotrophic conditions followed by mixotrophic conditions. The mixotrophic cultivation gives a biodiesel productivity that is higher than when cells are cultured under photoautotrophic conditions. Yeh & Chang [42] investigated both the growth and lipids productivity of *Chlorella Vulgaris ESP-31* under different growth metabolisms for various culture media. Results showed that under mixotrophic conditions, both lipids content and productivity are enhanced while compared to the other cultivation conditions. Table 2-1 reports a comparison between microalgae growth conditions based on the source of energy or based on carbon used.

Table 2-1 Summary of Microalgae Cultivation Conditions- Adapted from Yeh & Chang [41]

Cultivation Condition	Energy Source	Carbon Source
Phototrophic	Light	Inorganic Carbon
Heterotrophic	Organic Carbon	Organic Carbon
Photoheterotrophic	Light	Organic Carbon
Mixotrophic	Light and Organic Carbon	Inorganic and Organic Carbon

2.5.2. Nutrients Concentration

Carbon, nitrogen, and phosphorus are generally used by microorganisms as a source of food. This is necessary for their life sustainability [20]. Therefore, microalgae can grow using these type of substrates too. In addition, many microalgae species have the adaptability to change from photoautotrophic growth to heterotrophic growth. This can be accomplished with the change of the nutrient carbon source [24].

Carbon

As described earlier, inorganic carbon can be used as a carbon source under phototrophic and mixotrophic conditions. In addition, the organic carbon found in nutrients can be used as a carbon source for both the growth of the microalgae and as an energy source for it. The specific role of the organic carbon depends strongly on the microalgae metabolism. This ability of the microalgae to use the organic carbon matter is especially relevant when microalgae are cultivated in wastewaters under mixotrophic conditions. This becomes, an important factor for very effective wastewater treatment processes with significant yields [54].

Zhu et al. [55] investigated the growth of *Chlorella Zofingiensis* in integrated fresh water and piggery wastewater (animal wastewaters) using tubular photobioreactors. The results obtained show that by increasing the initial COD concentration, which provides a measure of the organic compound present, the growth rate of the microalgal biomass augmented. Perez-Garcia et al. [48] reviewed the metabolism of glucose, glycerol, acetate, and other carbon sources for heterotrophic microalgae cultivation. These authors showed the significant flexibility of microalgae to various carbon sources media.

Although there was high biomass productivity under mixotrophic conditions, one can also notice that there was less CO_2 capture. However, in such operating conditions, both organic carbon and CO_2 are utilized as part of the cell body. This competition for carbon sources may reduce as a result, the CO_2 amount consumed by microalgae.

Nitrogen

Nitrogen compounds especially ammonium (NH_4^+) and nitrate (NO_3^-) are important substrates for microalgae growth. These compounds contribute to more than 10% of the microalgal biomass. Additionally, urea and nitrite are other forms of nitrogen compounds but the latter is considered toxic at high concentrations [24]. In particular, ammonium is preferred by microalgal cells. Hence, if ammonium exists in the culture medium, there will be no tendency to assimilate these nitrogen sources [24], [41].

However, some researchers claim that ammonium can also be toxic to microalgae. This is the case, if ammonium is present in high concentrations. Moreover, ammonia may also cause harmful effects to the environment, if it is directly released into the atmosphere. Thus, a proper selection of a nitrogen source is required for microalgae growth [6]. One can also notice that in the case of combined wastewater treatment and microalgal culture, the nutrients or the nitrogen sources may be difficult to be controlled. Hence, the aim is to remove these substances by utilizing them as a source of food, whatever their composition may be.

Microalgae convert inorganic nitrogen compounds such as ammonium, nitrate, nitrite, ammonia to nitrogen containing organic compounds like proteins, enzymes, chlorophylls through assimilation processes [41]. The metabolisms of carbon and nitrogen can contribute to microalgae cell growth [48]. In order to understand how organic carbon and nitrogen are integrated and used for cells growth, one should consider both assimilation and partitioning processes [56],

[57]. Assimilation refers to the uptake of nutrients while partitioning designates the cell separation or refers to the cell division process.

In this respect, Perez-Garcia et al. [48] studied ammonium, nitrate, nitrite, urea, and organic nitrogen assimilation processes for microalgae cultivated under heterotrophic conditions. In addition, other researchers [58]–[60] have focused on the effects of nitrogen concentration on cell growth in different media. A detailed discussion about the role of nitrogen chemical species concentration for biomass maximization and for how it affects the lipids content and composition is provided in the upcoming sections.

Phosphorus

Phosphorus is another important nutrient for microalgae growth. Phosphorus can participate in the formation of proteins, lipids, and intermediates of carbohydrates. Similarly, microalgae can incorporate inorganic phosphate compounds such as hydrogen phosphates ($H_2PO_4^-$and HPO_4^{2-}), forming organic species via phosphorylation. Furthermore, some microalgae are capable of utilizing phosphorus, forming organic esters which are valuable for cell growth [41]. At a relatively high pH media, phosphates sediment in the formof phosophoric salts and organic matter [61].

Microalgae cells tend to store the excess amounts of phosphorus as polyphosphate granules. Thus, these species can be used by microalgae during phosphate starvation conditions for cell growth [24]. As a result, the reduction of phosphates may affect the photosynthesis process and the lipids production [6]. In this respect,

the effects of initial phosphorus concentration on biomass productivity and lipids accumulation was studied by Belotti et al. [60] and Xin et al. 2010 [62].

Other Micronutrients/Elements

Other micronutrients like trace metals such as silicon and iron may be used by microalgae culture. These species may be toxic to some microalgae species, at fairly high concentrations [41]. Thus, one can notice that some micronutrients may not critical for cell growth not having the same importance as carbon, nitrogen and phosphorus.

2.5.3. CO_2 Concentration and CO_2 Uptake Efficiency

Carbon dioxide is primarily used as a source of carbon for photoautotrophic microalgae cultivation. Over the past years, many researchers have studied the effect of CO_2 concentration on microalgae cell growth from a physiological view point. However, and in spite of the interest in this topic, there is still modest research developed to analyze CO_2 uptake under relevant process conditions [63].

Atmospheric air contains small amounts of CO_2. This CO_2 is not enough for microalgae cell growth due to the limited mass transfer driving forces. This problem can be solved by either taking CO_2 from a pure source or using CO_2 from flue gases, solving at the same time the environmental issue with CO_2 capture [64]. Additionally, when a CO_2 stream is fed to the culture media with spargers, large bubble formation reduces the efficiency of CO_2 utilization. Hence, small bubbles flows have to be implemented for better CO_2 uptake [30].

S.-Y. Chiu et al. [65] studied the growth of *Nannochloropsisoculata* in a semi-continuous culture. These results show that when air with 2v% CO_2 is fed to the culture medium, the growth of microalgae cells is stimulated. If however, higher concentrations of CO_2 are used in the culture medium (5 – 15 v%), this has negative effects on the cell growth.

Regarding culture media that contain phosphorus compounds, the addition of CO_2 decreases the pH of the media and this causes sedimentation of phosphorus compounds [55], [61].

In addition, Tang et al. [66] investigated the effects of different CO_2 concentrations on two microalgae species: *Scenedesmusobliquus* and *Chlorella Pyrenoidosa* as illustrated in Figure 2-3. It was observed, that biomass yield increases with CO_2 content at low CO_2 concentrations. On the other hand, biomass yield starts decreasing at higher CO_2 concentrations. These authors thus, identified 5-10% CO_2 as an optimum concentration range for microalgae growth.

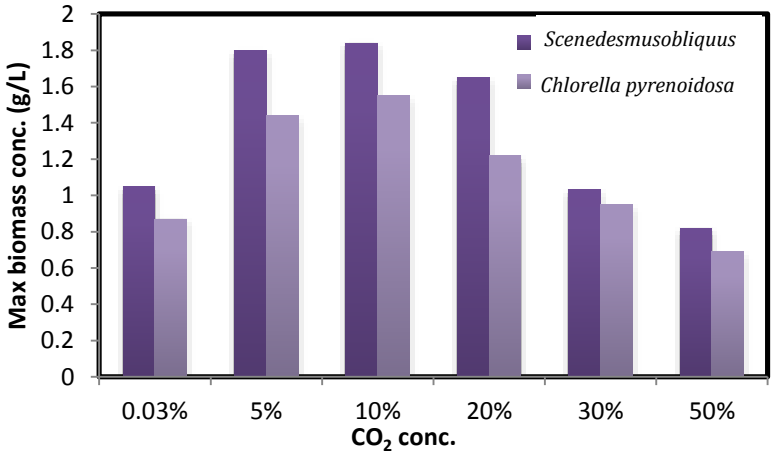


Figure 2-3 Effects of CO2 Concentration on the Biomass Concentration Produced. Data from Tang et al. [65]

2.5.4. Light and Photosynthetic Efficiency

The availability and amount of light is an important factor of the photosynthesis process on the microalgae growth. In this respect, one could mention that there are many parameters that affect the efficiency of light utilization such as the density of the culture and the cell pigmentation [67].

In open and outdoor cultivation systems, sunlight is directly applied to the culture media. Thus, in open and outdoor systems, one can notice that, there are at times, growth limitations given that light radiation is low and unequally distributed. On the other hand, in indoor lab scale closed systems, photobioreactors can deliver a much higher microalgae growth rate using fluorescent lamps. This artificial irradiation source provides higher density of radiation with an overall modest productivity of biomass [44]. Thus, the microalgae scale-up production, still requires the development and the implementation of sustainable radiation sources of light.

Rubio et al. [67] developed a model to describe the effects of light radiation on the photosynthesis process of microalgae growth. It was observed that to achieve high lipids production, light utilization should be enhanced to improve the photosynthetic efficiency. Thus, density of radiation and radiation utilization efficiency are important factors in algae culture, in addition to others, such as proper selection of microalgae strains and other growth parameters [13].

2.5.5. Culture Temperature

Temperature has noticeable effects on the microalgae growth and biomass production, because it affects the metabolic process and the biological reaction rate[30], [68].

Seasonal and daily fluctuations of weather conditions make it difficult to control the temperature within a specific range for outdoor microalgae cultivation. This is especially true, in colder days, when the relatively low temperature affects the microalgae growth. This problem can be addressed by developing microalgae culture in conjunction with a relatively warm CO_2 source. On the other hand, in summer days when the temperature is very high, evaporative cooling can be used to favour the best conditions for microalgae culturing. This can be achieved by spraying water into the cultivation tank [30].

Indoor cultures have the advantage of better allowing temperature control at desired thermal levels. Therefore, by placing the photobioreactor in a special room with a set temperature, the microalgae medium can be kept at a set temperature [39].

2.5.6. Culture pH

The pH of the culture media is an important factor affecting algae growth. This is the case since inappropriate (high or low) pH may have a negative impact on microalgae cell growth. Usually, acidic media (pH 5–7) is favorable for the growth of freshwater eukaryotic algae while alkaline media (pH 7–9) is beneficial for the growth of cyanobacteria (blue-green algae) [69]. However, decreasing the pH of

culture media, leads to sedimentation of phosphorus compounds, reducing the utilization of phosphorus media compounds [55], [61].

2.5.7. Effect of the Gas Flow Rate in Culture Mixing

Free atmospheric air mixed with pure CO_2 or even flue gases, can be fed to a microalgae culture. By using adequate flows, this may help prevent both nutrient and the cell precipitation, enhancing the cell growth.

Indeed, due to mass transport limitation and CO_2 diffusion through the liquid media, increasing gas flow rate helps to maximize biomass productivity. It is found that in many photobioreactors, gas is introduced to provide both turbulence and keep microalgae cells in suspension [30]. In fact, the thickness of the water layer surrounding the cells of the microalgae determines the rate of nutrient transfer or utilization. Thus, once turbulence is created in the culture media, favorable rates of nutrient transfer are secured [24].

2.6. Microalgae Biomass Harvesting

The harvesting of microalgae biomass is an operation that involves the separation of the biomass from the water media. This process is required because the microalgae culture media contains a high amount of water. The harvesting is normally performed using different solid-liquid separation processes. The specific separation processes and extent of separation can differ quite considerably from one microalgae species to another[13]. One can notice in this respect that, there are still difficulties in developing and selecting suitable harvesting microalgae

devices. Therefore, considerable research is being conducted nowadays to develop efficient technologies for microalgae biomass harvesting [70].

Indeed, it is difficult to find a harvesting process that offers a satisfactory performance and is economically feasible. Thus, to reduce the biomass harvesting cost, biomass can be concentrated during cultivation using biofilms [40].

2.6.1.Dewatering Processes

The dewatering process is aimed at removing a considerable amount of water from the microalgae culture and producing wet biomass. Different separation processes and unit operations have been used to remove large amounts of water from the culture media. Typical examples of dewatering processes are coagulation and flocculation as preparatory steps before applying physical separation are reported in [34].

A comparative evaluation between different microalgae harvesting methods by dewatering is reported in Table 2-2. Among them, discrete sedimentation, bioflocculation and autoflocculation are the less expensive ones in terms of operation cost and energy input. These processes however, strongly depend on the algae characteristics, with these characteristics strongly influencing separation time. On the other hand, centrifugation and chemical flocculation are less dependent on algae properties, providing a much faster separation. These methods involve, however, higher energy and operation costs.

Table 2-2 Comparison between Different Microalgae Dewatering Technologies - Adapted from Benemann & Oswald [39]

Process	Main Mechanism	Dependence on algae	Solid Conc.	Relative Cost	Energy Input
Centrifugation	Accelerated Separation	Minor	> 10%	High	High
Chemical Flocculation	Chemically Induced Floc	Minor	8 – 10 %	Medium - High	Medium - High
Cross Flow Filtration	Membrane Self-Cleaning	Minor	2 – 6 %	Medium	High
Micro-Straining	Screening	High	2 – 4 %	Low	Medium
Discrete Sedimentation	Gravity Discrete Settling	High	1 – 3 %	Low	Low
Bioflocculation	Spontaneous Flocculation	High	1 – 3 %	Low	Low
Autoflocculation	Ca/Mg ppt. Induced Flocs	Minor	1 – 3 %	Low	Low

2.6.2. Biomass Thermal Drying

The drying of microalgae biomass is needed for an high efficiency biodiesel production. This is the case given that the content of water in the biomass affects its preservation [34].

Thermal drying using solar energy can be used for drying the harvested wet biomass. However, its application is limited to locations receiving significant sunlight irradiation. Furthermore, in closed systems, alternative heat sources may also be used for drying [27]. Biomass drying has to be implemented, however, very carefully. Drying at elevated temperatures may be detrimental for lipids content and composition. Ali et al. [71], in their investigation on the thermal stability of microalgal biomass using *Nannochloropsis oculta* and *Chlorella vulgaris*, found that microalgal biomass starts to decompose at 220°C, while the maximum weight loss occurs between 277°C and 288 °C . This thermal level is a function of the heating rate.

2.6.3. Freeze Drying

In freeze drying, also known as lyophilisation, the concentrated biomass is frozen by reducing the algae temperature to around -40 °C, below the triple point as shown in Figure 2-4. At this temperature, most of the water is converted into ice.

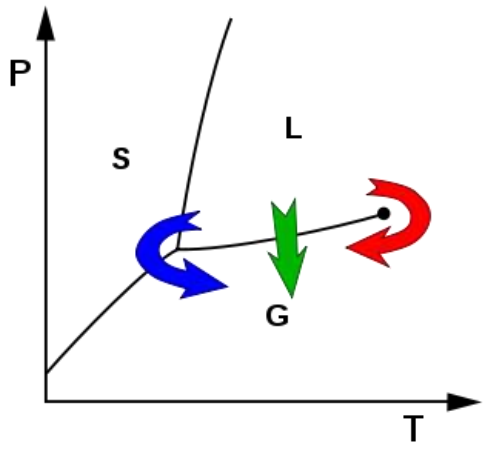


Figure 2-4 Iluustration of Freez Dring Through Phase Diagram

Figure 2-4 describes a water phase equilibrium showing the boundaries between gas, liquid and solid phases. In particular, phase changes from the triple point to the critical point are reported. Freeze-drying (blue arrow) brings the system around the triple point, avoiding the direct liquid-gas transition observed in ordinary drying operations (green arrow) [72]. This allows a primary drying sublimation to take place. Following this, a secondary drying could be implemented at relatively more elevated temperatures to remove the non-frozen absorbed water [73], [74]. Through this process, the harvested dried microalgae can be preserved for a long time. This drying process is however, unsuitable for industrial scale lipids

production. However, it can be used for other industrial applications such as cosmetics, medical drugs and protein supplement food.

2.7. Major Components of Microalgal Biomass

The resulting biomass from microalgae cultivation is very rich in some special components such as lipids. Lipids can be converted into biodiesel via well selected chemical processes. In addition, microalgae biomass contains other useful components like proteins. The residual biomass remaining from lipids extraction, can, hence, be used to produce other bio-products like ethanol by fermentation. It can also simply be used as feed or fertilizer [35]. In addition, it can possibly be converted thermally to biogas products.

Microalgae biomass mainly consist of lipids, proteins, carbohydrates and other inorganic species. The fraction of each component differs from one microalgae to another. It also depends on the cultivation conditions. One should notice that the specific composition of the microalgae biomass used(check)determines is actual value [75].

2.7.1. Lipids

Lipids are defined as any cellular compound that can be extracted by an organic solvent. Lipids are responsible for the fluidity of the membranes. Lipids can be classified into polar and non-polar. The polar lipids (membrane lipids) are present in the algae membranes in the form of phospholipids and glycolipids. On the other hand, non-polar lipids (neutral lipids) in algae are mainly composed of

triglycerides besides other components like pigments, vitamins, and hydrocarbons [40], [75]. Table 2-3 lists the reported lipids contents of different microalgae species.

Lipids molecules mainly consist of fatty acids, which are involved in both polar and neutral lipids. Fatty acids consist of a hydrophilic carboxyl group attached to a long saturated or unsaturated hydrophobic hydrocarbon chain [17]. Triacylglyceride or Triglyceride (TAG), sometimes referred to as triacylglycerol are important lipids components for biodiesel production. TAG molecules encompass fatty acids attached to glycerol units. A schematic of the TAG molecule is reported in Figure 2-5.a. A detailed discussion about lipids constituents and fatty acids composition will be provided later on in this review.

However, maximizing the lipids content and productivity is required, to enhance the biodiesel production. In addition, increasing the TAG fraction in the lipids, could reflect positively on the biodiesel production.

Table 2-3 Some Microalgae Species with High Lipids Content – Adapted from Chisti [12]

Microalgae specie	Oil content (% dry wt.)
Botryococcusbraunii	25–75
Nannochloropsissp.	31–68
Neochlorisoleoabundans	35–54
Nitzschiasp.	45–47
Schizochytriumsp.	50–77

2.7.2. Proteins

Proteins play a major metabolic and structural role in microalgae. Metabolic functions can be described as catalytic processes, enabling microalgae to grow. The structural role of protein can be visualized as one of providing scaffolds upon which the chlorophyll molecules are assembled under visible light, harvesting chloroplast complexes [75].

Proteins mainly consist of different amino acids. Therefore, the nutritional value of proteins depends on the composition and the content it its amino acids [76]. Typical structures of amino acids involved in microalgae are reported in Figure 2-5.b. One should notice that there are applications of microalgae as source proteins for human nutrition, animal feed and even cosmetics [77]. Table 2-4 lists the reported protein content of different microalgae species.

Table 2-4 Some Microalgae Species with Protein Content – Adapted from Becker [76].

Microalgae specie	Protein content (% dry wt.)
Anabaena Cylindrica	43–56
Aphanizomenonflos-Aquae	62
Chlamydomonasrheinhardii	48
Chlorella Pyrenoidosa	57
Chlorella Vulgaris	51–58
Dunaliellasalina	57
Euglena Gracili	39–61
Scenedesmusobliquus	50–56
Arthrospira Maxima	60–71
Spirulinaplatensis	46–63
Synechococcus sp.	63

2.7.3. Carbohydrates

Carbohydrates in microalgae can be present in different chemical forms such as sugars, glucose, starch, and other polysaccharides [77]. The structures of glucose and simple starches are described in Figure 2-5c&d. Microalgae microorganisms contain different types of monomers and polymer carbohydrate components. Carbohydrates also have both structural and metabolic roles in the synthesis of other algae biochemical substances [75].

Microalgae species can produce considerable amounts of carbohydrates. As a result, microalgae can be considered a valuable commodity. Thus, carbohydrate production from microalgae is a area offering significant commercialization opportunities [78]. Table 2-5 reports the carbohydrate content for several microalgae strains.

Table 2-5 Some Microalgae Species with High Carbohydrate Content – Adapted from Becker [76].

Microalgae species	Carbohydrates content (% dry wt.)
Anabaena Cylindrica	25–30
Aphanizomenonflos-Aquae	23
Chlorella Pyrenoidosa	26
Dunaliellasalina	32
Porphyridiumcruentum	40–57
Spirogyra Sp.	33–64

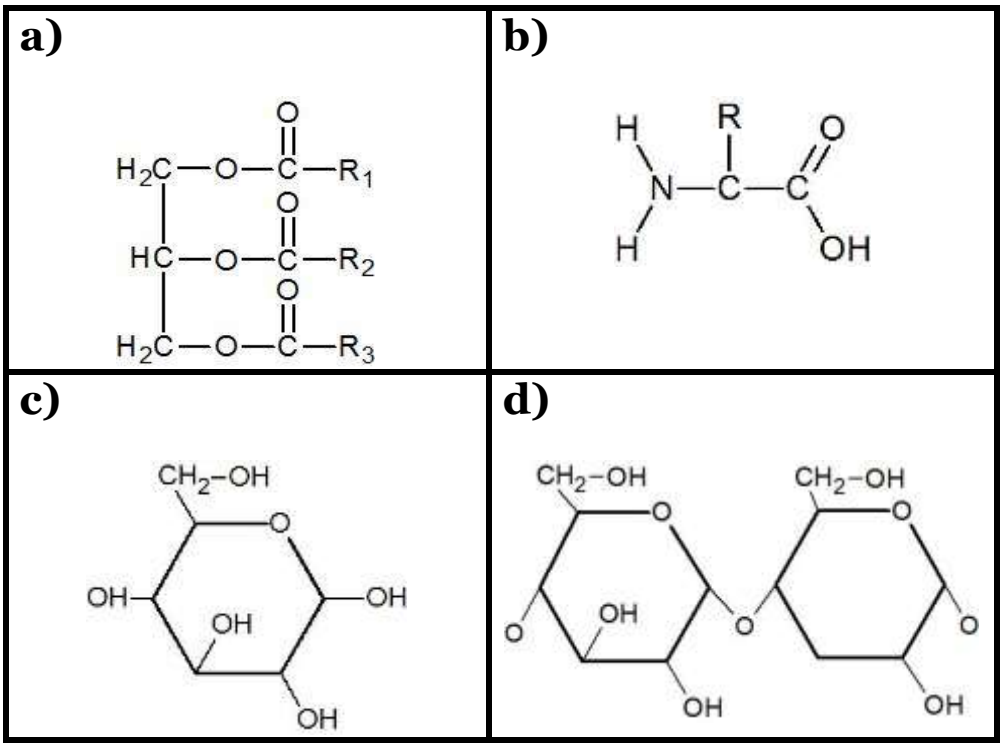


Figure 2-5 a) Triglycerides (TAG), b) Amino acid, c) Glucose, d) Starch

2.8. Methods of Biomass Conversion

Generally, biomass appears as a sustainable energy source that could replace the use of fossil fuels in the future. However, much research has still to be focused on the improvement of the quality and the quantity of biomass production as well as the development of efficient conversion technologies for biofuel production [79].

There are different conversion technologies for microalgal biomass processing. Each of them has advantages and disadvantages. Some of them, require further

research and investigation [80]. In addition, microalgae can produce non-fuel products that can be used as food supplements as well as for the pharmaceutical industry and other applications [81].

Biomass conversion processes can be classified into three main groups: (i) thermal methods(thermochemical), (ii) biochemical methods, and (iii) chemical methods, as illustrated in Figure 2-6.

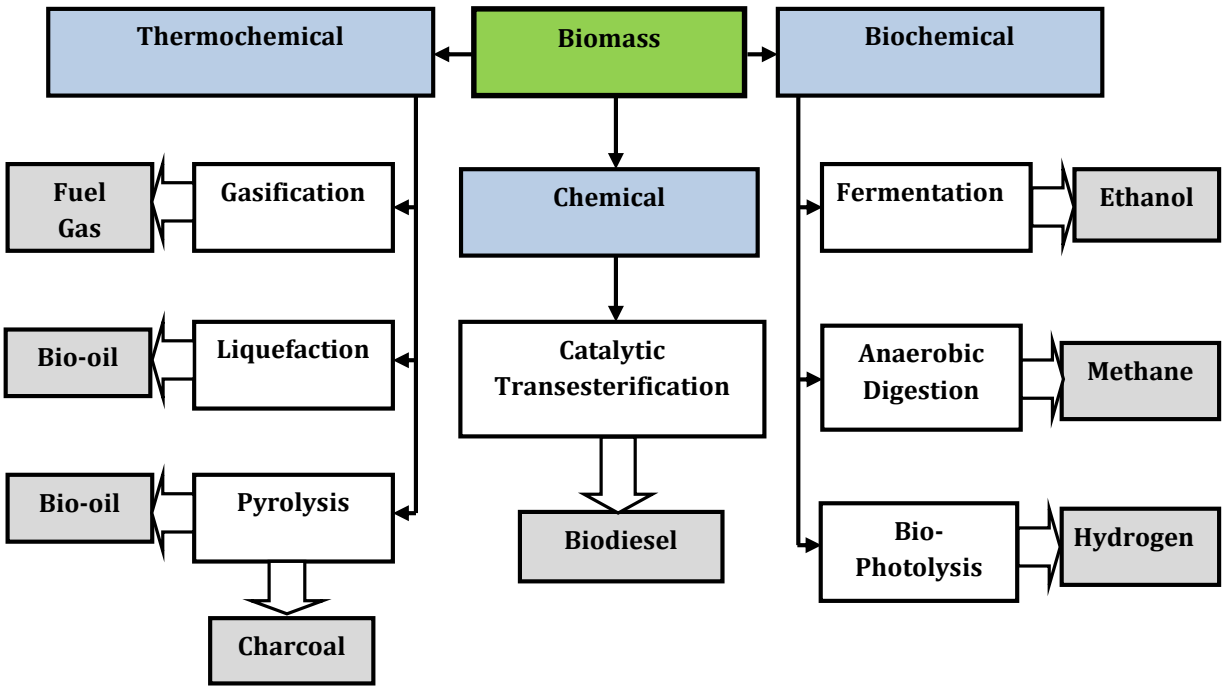


Figure 2-6 Methods of Microalgal Biomass Conversion Wang et al. [6]

2.9. Chemical Reaction Conversion

Over the past few years, due to the increasing price of biodiesel derived from crude oil, together with environmental concerns, alternative sources of biodiesel oil have been used and proposed using different crops and plants oil. However, there are some issue related to their sustainability and the lands required for cultivation.

On the other hand, microalgae have the potential of being a source of biomass production with high lipids content for biodiesel production. Recently, this topic has received great scrutiny. Another issue is the availability of land for the cultivation of microalgae oil for biodiesel production. This should occur without affecting the cultivation of other crops and creating food scarcity in those regions where microalgae cultivation for biodiesel production is taking place [69].

The extraction of lipids from microalgal biomass is, to some extent difficult, as result of the thick walls surrounding the cells which hinder the lipids from diffusing out of the cells. Mechanical pressure has revealed as not being very effective for microalgal lipids extraction, in comparison with oil extraction, from other terrestrial plants or crops [27]. Organic solvents and supercritical fluids can also be employed for microalgal lipids extraction. In this type of process, the following steps are considered: i) biomass is first pretreated, to prepare it for the lipids extraction process, ii) lipids extraction is accomplished using an organic solvent or supercritical fluid, iii) the produced liquids are separated from cell debris, by solid–liquid separation units, iv) the solvent and residual water are separated from the lipids using a liquid–liquid separation, v) the recovered organic solvent is

recycled back to the process. One should note that in the case of supercritical fluid extraction (e.g. case of using supercritical CO_2), both the fluid and residual water are in the gas state. Thus, the crude lipids are precipitated in a collection vessel [17].

Following this, biodiesel is produced via the transesterification of lipids. In transesterification, triglyceride lipids react with short chain alcohols such as methanol or ethanol in the presence of a homogeneous or heterogeneous catalyst [27]. After transesterification is completed, the resulting mixture contains fatty acid methyl ester (FAME) or biodiesel, glycerol, a reformed alkali catalyst (if homogeneous alkali catalyst is used), excess alcohol (methanol), and unreacted lipids. In the next step, the liquid mixture is subjected to a post-transesterification purification, in order to recover the biodiesel fraction and to remove by-product contaminants[17]. Figure 2-7 provides a conceptual description of the biodiesel production processes including the various described steps.

Regarding the present review, the upcoming sections focus on the specific ways of converting the microalgal biomass to biodiesel. A detailed discussion about lipids extraction and lipids analysis is also be provided.

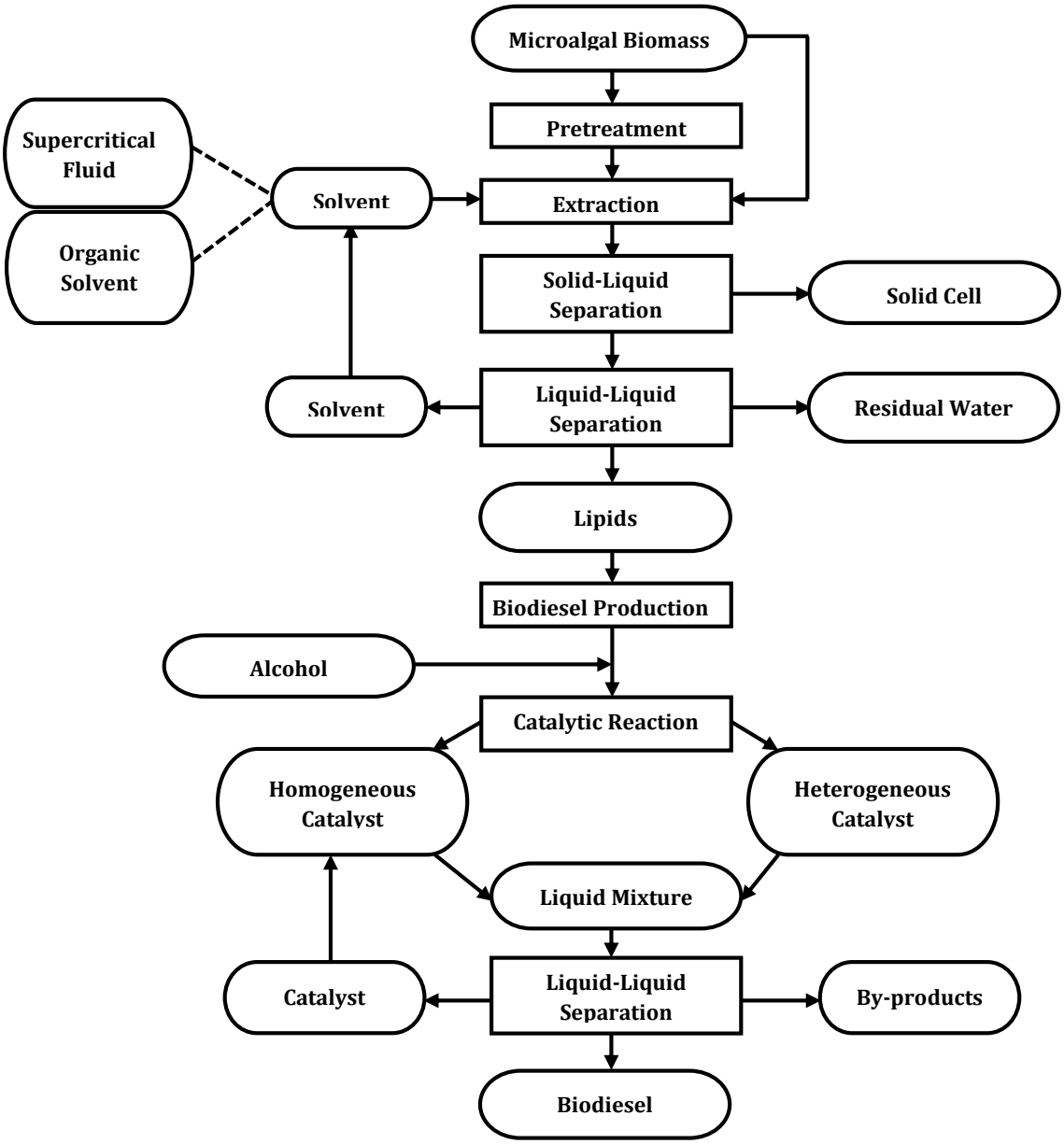


Figure 2-7 Microlagal Biomass Chemical Reaction Conversion Method

2.9.1. Cell Disruption Processes

Diffusion is considered to be the rate controlling factor in the overall mechanism of the extraction process. In this process, chemical solvents are used as extracting agents. Consequently, cell disruption can improve the efficiency of solvent diffusion, which promotes the lipids extraction [27]. Cell disruption or cell

breaking, avoids the need to operate the extraction process at a high temperature and pressure [82]. Several methods are proposed for cell disruption. These methods can be categorized into mechanical methods (such as bead mills, sonication, cavitation and autoclaving), and non-mechanical methods (such as freezing, osmotic shock, and chemical methods) [82].

2.9.2. Methods of Lipids Extraction

The extraction of lipids from microalgal biomass is an important step toward successful biodiesel production. Lipids extraction is a mass transfer operation, in which lipids get separated or are released out of the biomass cell walls. [83]. However, compared to oil extraction from other terrestrial energy crops, microalgae lipids extraction is more difficult. This is a consequence of the existence of the thick cell walls which hinder intra-lipids from diffusing out of the algae structure [27]. Therefore, extraction may strongly depend on the microalgae species, and on the thickness of their cell walls [82].

One should note as well that there are still challenges with the extraction of desired chemical species. Firstly, not all the lipids constituents can be used for biodiesel production. Thus, the extraction process has to be selective in order to extract the desirable lipids portion [80]. In addition, other biomass non–lipids components may also get extracted, with this non-lipids constituents fraction affecting the overall mass transfer during the extraction process.

The most common methods used for lipids extraction are mechanical extraction, chemical solvent extraction, and supercritical fluid extraction [84]. Chemical

solvent extraction is usually used, because it has economical and technical merits such as the low cost of solvents and the equipment utilized, with this making the scale-up possible [85]. In addition, the extraction methods can be classified into wet or dry extraction, depending on the amount of water in the biomass to be processed [86]. Pretreatment of dry microalgal biomass or cell disruption may increase the accessibility to the desired algae lipids. One could also mention that there are various mechanical and non-mechanical methods available for lipids extraction and specifically designed to account for the microalgae cell wall thicknesses in the algae product [34]

2.9.3. Organic Solvent Method

Lipids or oils are highly soluble in organic solvents [87]. Hence, lipids contain non-polar lipids (neutral lipids) which are hydrophobic molecules that interact with non-polar organic solvents (e.g., hexane, ethyl ether, chloroform and benzene). On the other hand, for polar lipids (membrane lipids), polar organic solvents (e.g., ethanol and methanol) are used to break the hydrogen bonding between the lipids and proteins [38], [88].

In fact, the selection of organic solvents, depends on the type of microalgae species used, as well as other preferred characteristics such as being inexpensive, highly volatile, insoluble in water, non-toxic and selective toward the extraction of the desired components [87], [88]. However, some organic solvents like *n*-hexane, methanol and chloroform are considered highly toxic solvents. Their usage should be carefully monitored because they may cause serious impacts on health [27]. On the other hand, ethanol as a solvent, offers a low level of toxicity. It can be produced

from various renewable sources. Nevertheless, the usage of ethanol, in the presence of water, may reduce the extraction efficiency. This is the case given that ethanol forms an azeotrope with water at specific water-ethanol concentrations [27].

More recently, there is a trend to use ionic liquids as co-solvents (with organic solvents) for microalgal lipids extraction. Ionic liquids have unique characteristics such as low toxicity and negligible vapor pressure. It is felt however, that more research is needed in this area [89], [90] before specific applications can be implemented.

Alternatively, Accelerated Solvent Extraction(ASE), which utilizes organic solvents at temperatures higher than their boiling point, has also being proposed to enhance the extraction process [87].

Commonly, there are two well-known methods for laboratory lipids extraction. The first method is the Soxhlet Extraction, which normally utilizes *n*-hexane as an extracting solvent. The second method is called the Bligh and Dyer Method wherein lipids extraction is performed by solvents consisting of a mixture of chloroform and methanol [38], [80].

2.9.4. Transesterification of Lipids

The transesterification method is widely used for the conversion of oil from natural sources like vegetables oil, terrestrial plants oil, algal oil, etc. into liquid fuel. In the transesterification reaction, lipids that contain mainly triglycerides, are reacted with short chain alcohol (e.g., methanol and ethanol) to produce biodiesel.

Biodiesel, a mixture of fatty acid alkyl esters, is the main product while glycerol is a by-product (refer to Figure 2-8). The transesterification reaction takes place in the presence of a catalyst [34], [38]. Commonly, methanol is used for the transesterification of lipids to produce biodiesel which consists of fatty acids methyl esters (FAMEs). The transesterification reaction can be classified depending on the catalyst state as homogenous or heterogeneous .

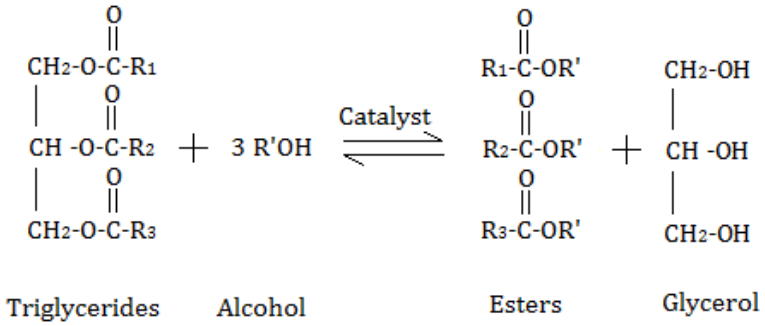


Figure 2-8 Transesterification of Lipids

2.10. Summary of the Literature Review

- Microalgae is a green technology for CO_2 capture. However, determination of the CO_2 bio-fixation rate was just based on measurement of CO_2 concentration at the reactor inlet and outlet, which is not very accurate. Therefore, a reliable method should be developed.

- Integration of microalgae culturing with wastewater treatment plant would help in removing major nutrients from wastewater discharge. Indeed, more researches are required to study the effects of variation on nutrient levels.

- The produced microalgal biomass can be further converted to valuable fuel and non-fuel products. In particularly, the thermal conversion of microalgal biomass and identification of reaction kinetics should be studied carefully.

- Some microalgae strains have such high oil yields, thus they are appealing candidates for next generation biodiesel production.

CHAPTER 3

THESIS OBJECTIVES

3.1. Overall Objective

The main objective of this research is to investigate the integrated CO_2 bio-fixation, wastewater treatment and biofuel production by cultivating two (*Nannochloropsis oculata* and *Chlorella vulgaris*) microalgae species. In this regard, the optimum conditions to be determined to achieve maximum CO_2 bio-fixation and nutrient removal from the waste water media.

3.2. Specific Objectives

The following are the specific objectives are identified to achieve the overall objective:

1. To develop a reliable method to determine the rate of CO_2 bio-fixation using microalgae, based on the measured carbon content of microalgae cells.

2. To study the rate CO_2 bio-fixation under phototrophic and mixotrophic conditions. The phototrophic represents a growth medium without organic carbon (CO_2 is the main source of carbon), which simulates the wastewater discharge from the secondary treatment unit. The mixotrophic cultivation accounts both CO_2 and organic content in the wastewater as nutrient

sources for microalgae cells. The mixotropic cultivation represents the growth in wastewater stream before the secondary treatment units.

3. To study the effects of different initial nutrients concentration on their removal from wastewater effluent using microalgae culturing.

4. To analyze the thermal stability of microalgal biomass upon heating, since such decision would help in setting temperature limits for biomass thermal drying and for high temperature oil (lipids) extraction. Also, for the thermal conversion of microalgae biomass for energy purposes.

5. To determine the amounts of lipids could be produced when microalgae cultivated in municipal wastewater medium.

6. To evaluate the effect of cells disruption on the efficiency of lipid extraction process.

CHAPTER 4

MATERIALS AND METHODS

In this chapter, types of microalgae species were mentioned. Additionally, the culture medium in which microalgae cultivated is fully described. Different analytical methods were used to monitor the cells growth, uptake of nutrients, and for characterizations of microalgae biomass. As well, the procedures for culturing microalgae and harvesting the produced biomass are clearly demonstrated.

4.1. Microorganism

Two microalgae strain were used in this study, *Nannochloropsis oculata* (*N. oculata*) and *Chlorella vulgaris* (*C. vulgaris*), which are fresh water microalgae. These strains were originally purchased from www.algaeDepot.com company.

4.2. Culture mediums

The phototrophic culture medium is the traditional Basal Bold medium (BBM) (see appendix A) which consist of (g/L): $NaNO_3$, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, K_2HPO_4, KH_2PO_4, NaCl, Alkaline EDTA, KOH, $FeSO_4.7H_2O$, H_2SO_4, H_3BO_3 and trace metal solution of $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, $CuSO_4.5H_2O$, and $CoCl_2.6H_2O$.

The mixotrophic culture medium was prepared by addition of glucose to Basal Bold medium (BBM). Glucose concentration in the medium was initially adjusted to 1 g/L.

A synthetic tertiary wastewater was prepared by modifying the traditional Basal Bold medium (MBBM). The prepared medium consist of (g/L): $CaCl_2.2H_2O$, $MgSO_4.7H_2O$, KH_2PO_4, NaCl, Alkaline EDTA, KOH, $FeSO_4.7H_2O$, H_2SO_4, H_3BO_3 and trace metal solution of $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, $CuSO_4.5H_2O$, and $CoCl_2.6H_2O$. Additionally NH_4CL was used as sole nitrogen source, and KH_2PO_4 as the only source of phosphorus. The concentration of ammonia NH_3 and phosphate PO_4^{3-} was adjusted to be within the range of typical municipal wastewater. Therefore, the NH_3 concentrations were between 20 to 75 mg/L and for PO_4^{3-} between 3 to 16 mg/L roughly. These limits were specified to account for the high and low strength municipal wastewater.

4.3. Experimental set-up

A given volume was taken from early prepared inoculums then added to a fresh medium. The culture was pre-cultured again for a few days, the flasks were covered with plastic covers, having two holes for gas inlet and outlet. After enough period, culture was centrifuged to remove the consumed medium, and then a new medium was added to achieve constant cell concentration for microalgae culturing initialization. To start the cultivation in batch reactor, Erlenmeyer flasks (0.5, 1 and 2 Liter) were used. The initial concentration was adjusted, and the flasks were placed in a fume hood. Fluorescent light provided to all the reactors from both

sides, while the temperature kept at ambient condition. CO_2 mixed with air using air-mixing device, and the gas stream continuously bubbled at the bottom of the reactor. CO_2 concentration in the gas stream was as adjusted to 4 %. While aeration was performed in parallel (see Figure 4-1). A summary of culture conditions for all experiments are listed in Table 4-1.

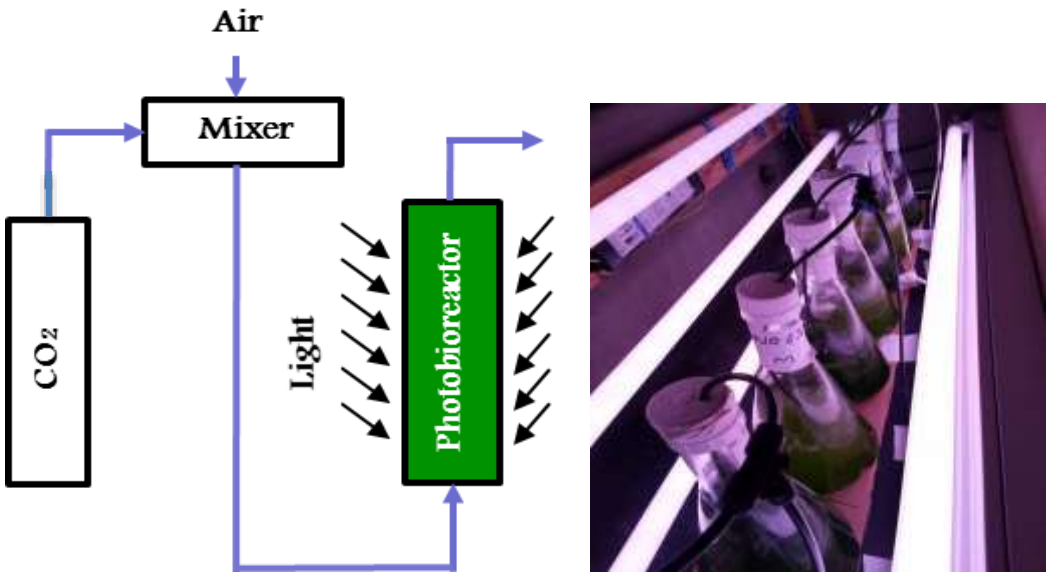


Figure 4-1 Experimental Set-up of Microalgae Culturing Unit.

Table 4-1 Summary of Microalgae Culturing Conditions

Materials and Methods	CO_2 Capture Investigation	Nutrients Uptake Investigation	Biomass Thermal Conversion	Lipids Content Investigation
Microalgae Species	*N. oculata,* *C. vulgaris*	*N. oculata,* *C. vulgaris*	*N. oculata,* *C. vulgaris*	*N. oculata,* *C. vulgaris*
Culture medium	BBM	MBBM	BBM	MBBM
TN-NH3 (mg/L)	-	20 - 75	-	52.8±0.9
TP- PO_4^{3-} (mg/L)	-	3 - 16	-	13.5±2.5
CO_2 conc. (%)	4	4	4	4
Initial volume (ml)	900	400	1700	1500
Gas flow rate (≈ cc/min)	5	5	-	-
Light intensity (Lux)	1600<	2800 - 5600	-	1400-2000
Temperature (℃)	22±2	22±2	-	-
Cultivation period (days)	10	8	10	7

4.4. Biomass Harvesting and Drying

At the end of cultivation period, the cultures were harvested by centrifugation at 9000 rpm for 3 min using High speed refrigerated centrifuge, HITACHI-CR22GIII (see Figure 4-2), and the wet biomass were collected. In order to dry the wet biomass, small volume of deionized water was added, followed by pre-freezing to -40℃ by using a deep freezer. Finally, biomass freeze-dried under vacuum and at low temperature of -80℃ using a VirTis freeze dryer - SP Scientifics - shown in Figure 4-3.

Figure 4-2 High Speed Refrigerated Centrifuge, HITACHI-CR22GIII

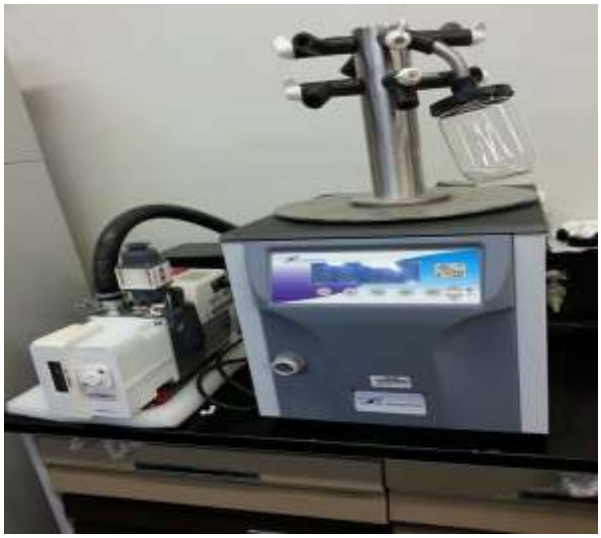


Figure 4-3 VirTis Freeze Dryer with Vacuum Pump

4.5. Analytical Methods

4.5.1. Dry Cell Weight Analysis

Dry cell weight analysis is an important test to estimate both microalgae concentration and microalgal culture productivity. When using this method, liquid samples are taken and then dried. Following this, their weights are measured. Then, given the sample volumes and their weights, microalgae concentrations are determined [91].

Since a small liquid sample is been used in the dry cell weight analysis, the sample employed should be selected carefully to be representative of the bulk algae culture. Furthermore, several methods can be used for dry weight measurements such as filtration, centrifugation, and turbidity. Each method has its advantages and its issues as reported by the Algal Organization Report [92].

In the present work, a given volume of aqueous sample was taken from cultivation reactor, and dewatered using vacuum filtration method (Figure 4-4). The filtered wet biomass was then dried in an oven at 50℃ overnight. Dry biomass weight determined gravimetrically.

Figure 4-4 Vacuum Filtration Method

4.5.2. Optical Density Measurement

Optical density was measured on a daily basis to monitor the cells growth. However, this colorimetric method was usually used to analyze the growth behavior based on the change in the color of the culture. The reading was recorded at a wavelength of 690 nm (OD_{690}) using UV-visible light spectrophotometer Evolution 260 BIO" - Thermo scientific - as shown in Figure 4-5. The absorbance at OD_{690} can be calibrated against dry biomass weight concentrations.

Figure 4-5 UV-visible light spectrophotometer

4.5.3. Total Nitrogen and Total Phosphorus Concentrations

Analysis of nutrients concentration was made by measuring Total Nitrogen (TN) in Ammonia (NH_3) form, and Total Phosphorus (TP) in the Phosphate form (PO_4^{3-}) concentrations in the culture medium every two days using a spectrophotometer (DR 3900 Bench-top Spectrophotometer) and digital reactor (DRB200: Digital Reactor) for coking as presented in Figure 4-6. The method used for TN-Ammonia analysis is termed as Salicylate Method, and for TP-Phosphate analysis is called as Molybdovanadate Method with Acid Persulphate Digestion.

For the TN-Ammonia measurement, sample volume of 0.1 ml is required. This small volume approximated as 2 drops using a plastic dropper (1 ml ≈22 drops). However, these TN values were then corrected using correlation between theoretical TN values and measured values obtained using solution of NH_4CL of

known TN-Ammonia. A correction factor of 1.215 with a correlation coefficient (R^2=0. 975) were obtained (See Appendix B)

a) **b)**

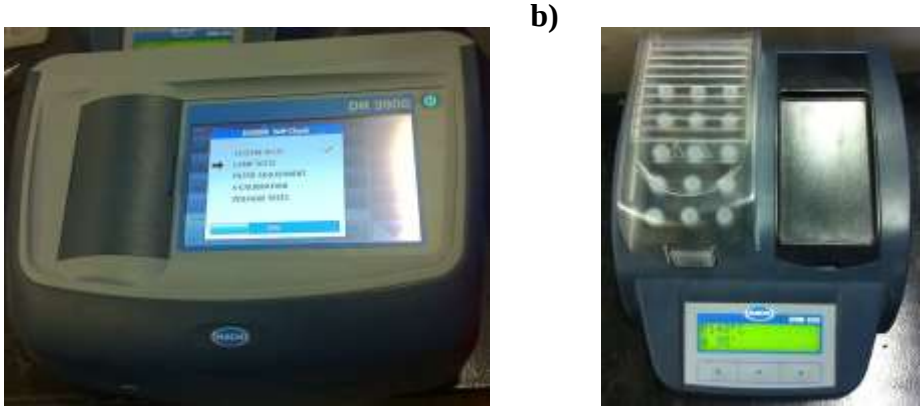


Figure 4-6 a) DR 3900 Bench-top Spectrophotometer, b) DRB200: Digital Reactor

4.5.4. Total Organic Carbon (TOC) Analysis

There are various TOC analyzers that may be used to measure the organic carbon content in liquid samples. TOC analysis can be also used for carbon content quantification, which helps in determining CO_2 biofixation rates.

Total organic carbon (TOC) was measured using the Torch TOC Analyzer (Teledyne Tekmar) as described in Figure 4-7. The TOC Analyzer calibrated with Potassium Hydrogen Phthalate (KHP) solution. Moreover, the calibration usually checked by Urea solution of a given concentration (Known TOC). The culture medium containing microalgae cells was subjected to the test every two days. The filtered medium (free of microalgae) was tasted too in the same manner.

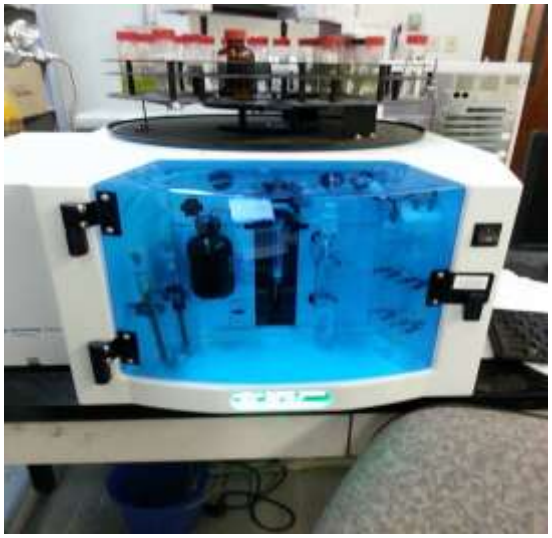


Figure 4-7 Torch TOC Analyzer

4.5.5. Thermogravimetric Analysis (TGA)

Thermal behavior characteristics of biomass can assist in obtaining reliable information required for the thermochemical conversion processes for fuel production. TGA analysis is widely used for investigating the thermal decomposition behavior of various materials [93], [94]. Thermogravimetric analysis of microalgal biomass gives valuable information about the initial decomposition temperature. This information helps considerably in setting the conditions for biomass thermal drying and subsequent processing using high temperature lipids extraction [71].

The high temperature thermal decomposition of microalgal biomass was evaluated using TGA (SDTG 600, TA Instruments, USA) as presented in Figure 4-8. For each experimental run, 6-6.2 mg biomass sample were placed on the sample holder. The samples were heated at air atmosphere with an air flow rate of 100 ml/min. The weight loss of the sample was recorded from ambient temperature to 800 ^{o}C

with heating rates of 5, 10, 15, 20 ^{o}C/min. Each experiment was repeated 2-4 times in order to confirm the minimum standard deviation of the percentage weight loss.

Figure 4-8 Thermogravimetric Instrument (SDTG 600)

4.5.6. Proximate and Ultimate Analysis

In order to determine the moisture content, the samples were exposed to constant temperature of 105 ^{o}C until stabilized their sample weight [95]. Following the moisture removal, the ash and other volatile matter fractions were estimated by modifying the traditional standard methods for biomass as described by Cantrell et al. [96].

For ash measurement, the sample was heated at 600 °C under air flow with heating rate of 11 ^{o}C/min and held at isotherm temperature for 10 min. In order to determine the amount of the volatile substance, the sample equilibrated at 110 ^{o}C then heated to 950 ^{o}C with 100 ^{o}C/min heating rate in nitrogen atmosphere and

held for 7 min. The gas flow rate for both test were kept at 80 ml/min. The fixed carbon content was calculated by difference. The elemental composition represented by the percentage weight of the carbon, hydrogen and oxygen were determined using correlations developed by Parikh et al. [97], while the corresponding higher heating value calculated as using correlation developed by Parikh et al. [98]. All correlations based on proximate analysis.

4.5.7. Lipids Extraction Using Soxhlet Method

In this method, the Soxhlet apparatus is used for extraction purposes. Firstly, the microalgal biomass, is fed into the extraction zone in a cellulose thimble (refer to Figure 4-9a). The bottom flask is filled with the solvent to provide the desired biomass/solvent ratio. Then, by continuous heating of the flask, the solvent evaporates. The evaporated solvent cools down in the upper condenser. The liquid solvent then moves, to the extraction zone, where it extracts oil from the biomass. Continuous solvent condensation, causes the liquid level to rise in the extraction zone until it reaches a pre-determined level. At this point, as a result of a siphon effect, the condensate starts overflowing from the extraction zone to the bottom flask. Given that the heating of the lower flask continues, the evaporation-condensation-extraction cycle repeats itself many times, with the solvent in the flask being progressively enriched by the microalgal extract. At some point, the process is interrupted and one is left with an extracted oil-solvent liquid phase in the bottom flask. However, and in order to obtain a highly concentrated extracted oil, further evaporation of the solvent from the extracted oil may still be required.

In the present work, Lipids extracted by using Soxhlet extractor EZ 30/H (See Figure 4-9b). However, *n*-hexane was used as an extraction solvent. Firstly, 50±5 mg of biomass sample was placed in a cellulose thimble (EX 30 HS). 80 ml volume of hexane was filled in the bottom of flask of the Soxhelt extractor. Heat was provided continuously to the bottom flask by adjusting the heater to level 4. In order to condense the evaporated solvent, cooling fluid 134-a is recirculated at the column upper side, the condenser temperature was maintained at 10℃. The solvent was left refluxing for 6 hr. After all lipid extracted, the bottom flasks containing the solvent and the lipids were removed. Then, hexane was recovered and the lipids completely dried. Finally, total crude lipids content determined gravimetrically. The test was repeated twice for each microalgal biomass, and the highest values of lipids content were reported.

a) **b)**

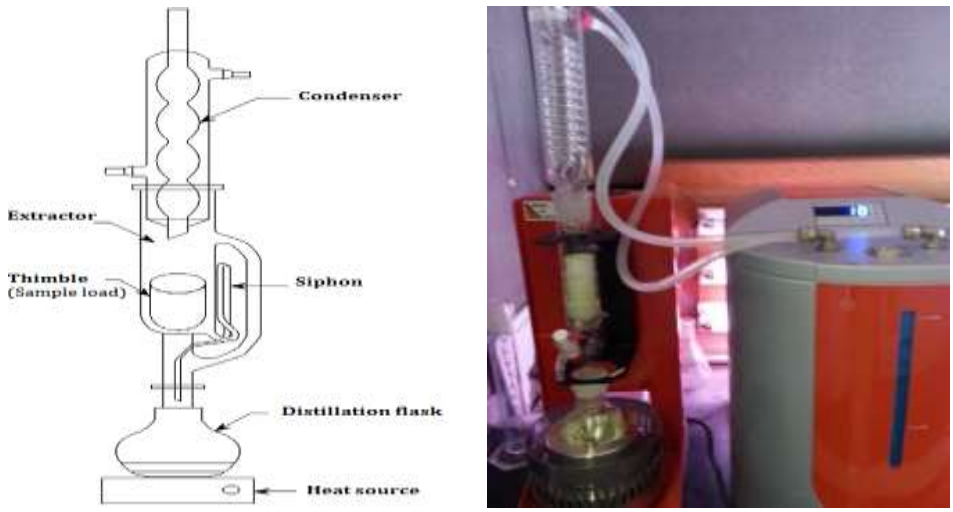


Figure 4-9 a) Schematic Representation of a Soxhlet Extractor Unit. Adapted from - (Dutta et al. 2014), b) Soxhlet Extractor EZ 30/H

4.5.8. Sonication Assisted Lipids Extraction

Ultrasound waves are defined as high frequency pressure waves at a decibel levels above human hearing (above 20 kHz) [99]. The propagation of these waves in the liquid, causes microbubble cavitations. The created cavitation shock waves lead to cell wall disruption or breakdown [17].

In the present work, 20 kHz QSONICA Sonicator (See Figure 4-10) was used. 30 ml of hexane was added, the biomass sample was sonicated for 30 min with 50 Amplitude, then the extracted oil filtered with a cellulose thimble. The biomass residue was further processed in Soxhelt extractor (80 ml total hexane volume) for 6 hr at the same condition described earlier. Again, at the end of extraction process the solvent was recovered and total crude lipids content determined gravimetrically. The test was repeated twice for each microalgal biomass, and the highest values of lipids content were reported.

Figure 4-10 QSONICA Sonicator (20 kHz).

RESULTS & DISCUSSION

CHAPTER 5

CO_2 CAPTURE USING MICROALGAE

In this chapter, a new reliable approach for determination of CO_2 capture by microalgae culturing was implemented. However, previous studies reported in the literature focus on measurement of CO_2 at the inlet and outlet of the reactor. Nevertheless, in this work we use the concept of carbon content analysis to estimate the amount of carbon dioxide consumed by microalgae cells during their growth. Additionally, quick comparisons between CO_2 captured under phototrophic and mixotrophic conditions, biomass yield and depletion of the organic carbon from the medium were presented. However, such information will help in decision-making related to the location of the algal treatment unit within the wastewater treatment plant.

5.1. Microalgae Cultivation Conditions

Microalgae was cultured under phototrophic and mixotrophic conditions. The phototrophic growth occurs when there is no organic carbon compounds in the culture medium [44]. Therefore, this situation is analogue to the effluent from the secondary treatment unit in the wastewater treatment process. While, the mixotrophic metabolism occurs when there is an organic carbon source present in the medium, therefore both CO_2 and the organic compound are used by cells as carbon sources [34]. Therefore, this mixotrophic cultivation is simulate a wastewater stream before the secondary treatment unit.

5.2. Microalgae Cells Growth

Figure 5-1 shows the change in optical density over time for *N. oculata* and *C. vulgaris* cultured under phototrophic and mixotrophic conditions. Figure 5-2 describes the biomass growth curves for *N. oculata* and *C. vulgaris* for the two cultivation modes. From Figure 5-2a *N. oculata* almost shows a similar trend for phototrophic and mixotrophic conditions. Hence, initially there was a slight difference between their dry biomass weight values, but at the end of the cultivation period they almost give the same value. On the other hands, for C. *vulgaris* at the beginning there was no significance difference between the phototrophic growth and the mixotrophic growth. Nevertheless, at the final culturing date, phototrophic condition results in higher biomass yields than mixotrophic mode as described in Figure 5-2b. While, in term of optical density it is very clear the gap between phototrophic and mixotrophic absorbance curves as shown in Figure 5-1a&b for *N. oculata* and *C. vulgaris*.

In addition, it was observed that starting from day 3, microalgae cultured under mixotrophic condition by addition of glucose, it has begun to agglomerate and to form bigger flocs at the bottom, and almost clear liquid at the top of the reactor. However, this might be due to the action of bacteria, since the medium was not sterilized initially.

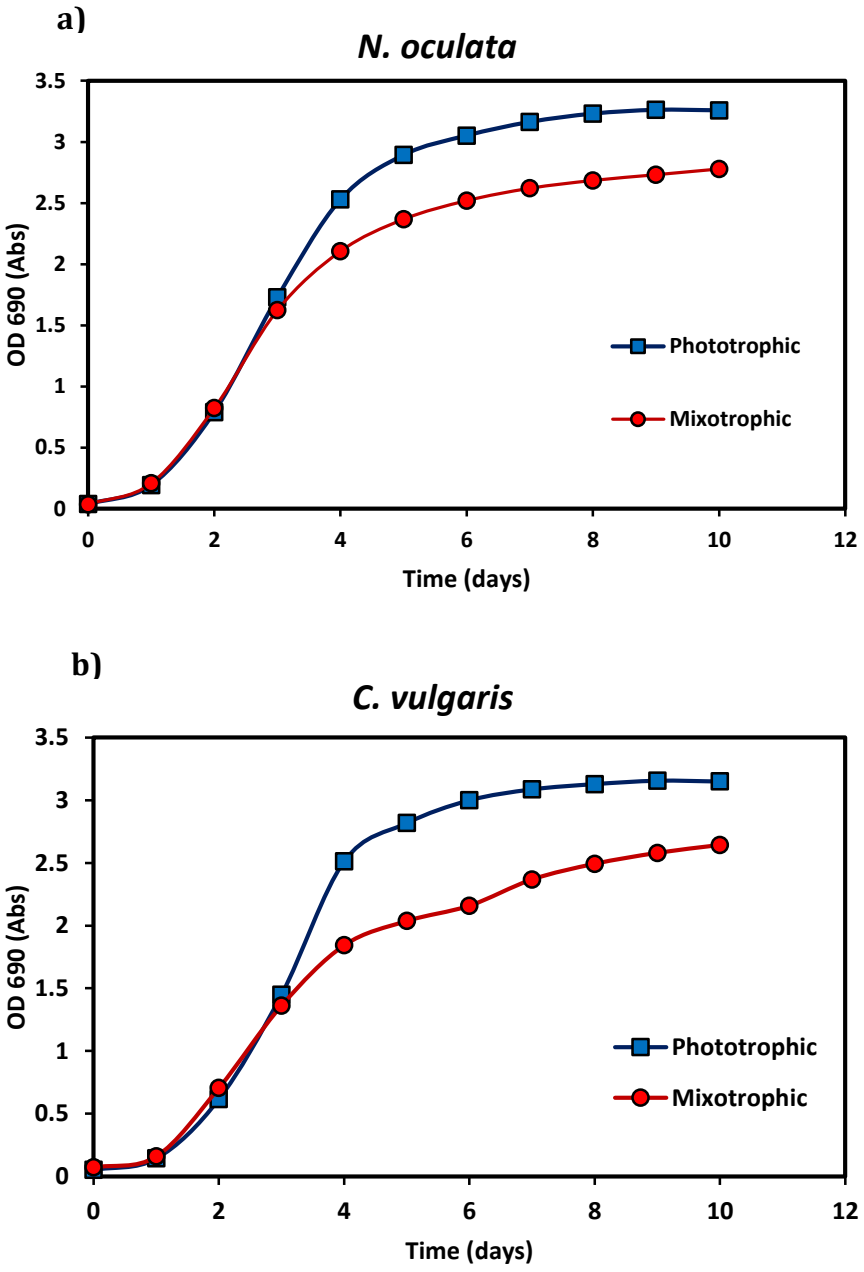


Figure 5-1 Changes in Culture Optical Density a) *N. oculata*. b) *C. vulgaris*.

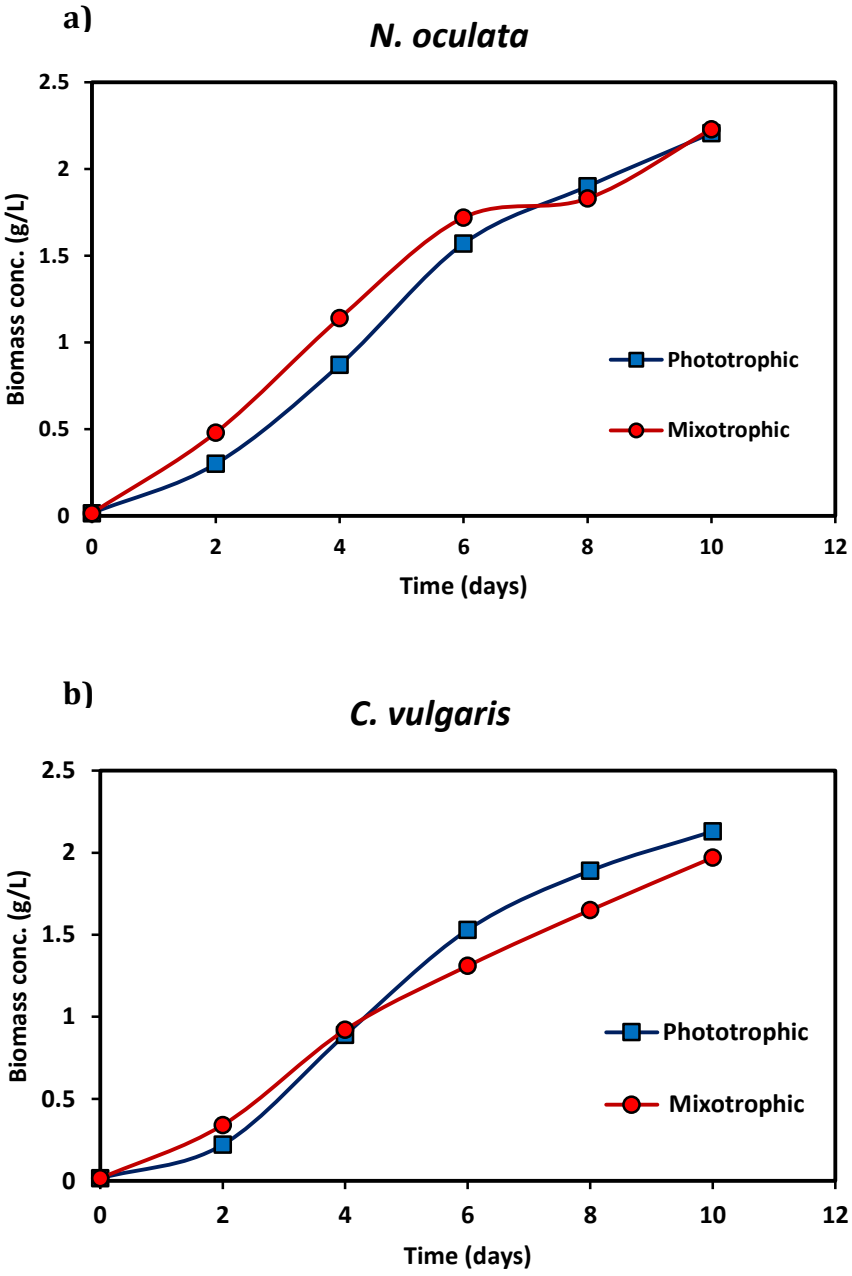


Figure 5-2 Microalgae Cells Growth curves of a) *N. oculata*, b) *C. vulgaris*

5.3. Carbon Content of Microalgae

As a matter of facts, the total organic carbon of microalgae cells increase with culturing duration, due to the increase in the number of cells produced. Though, for *N. oculata* cultured in phototrophic condition the TOC of the microalgae cells is much higher than for mixotrophic metabolism as clear in Figure 5-3a. However, this result indicates that under phototrophic condition cells accumulate more organic carbon than the other condition. Therefore, microalgae prefer inorganic carbon source (CO_2) as a food than organic carbon source (glucose).

Meanwhile, the results of *C. vulgaris* confirmed this postulation. Since there is a wide gap between TOC values for microalgae cultured under the two modes mentioned earlier. Hence, from Figure 5-3b at day 2 the TOC for both conditions are exact value. Nevertheless, by day 10, the TOC for phototrophic mode is around 1136 ppm, while for mixotrophic mode is just 678 roughly.

Although, the carbon content can account for CO_2 fixation rate, but this is true if the culture medium does not contain any carbon source rather than CO_2. Hence, if CO_2 is not the only source of carbon for microalgae cell to grow, then a carbon balance should be made to quantify the carbon content obtained from just CO_2.

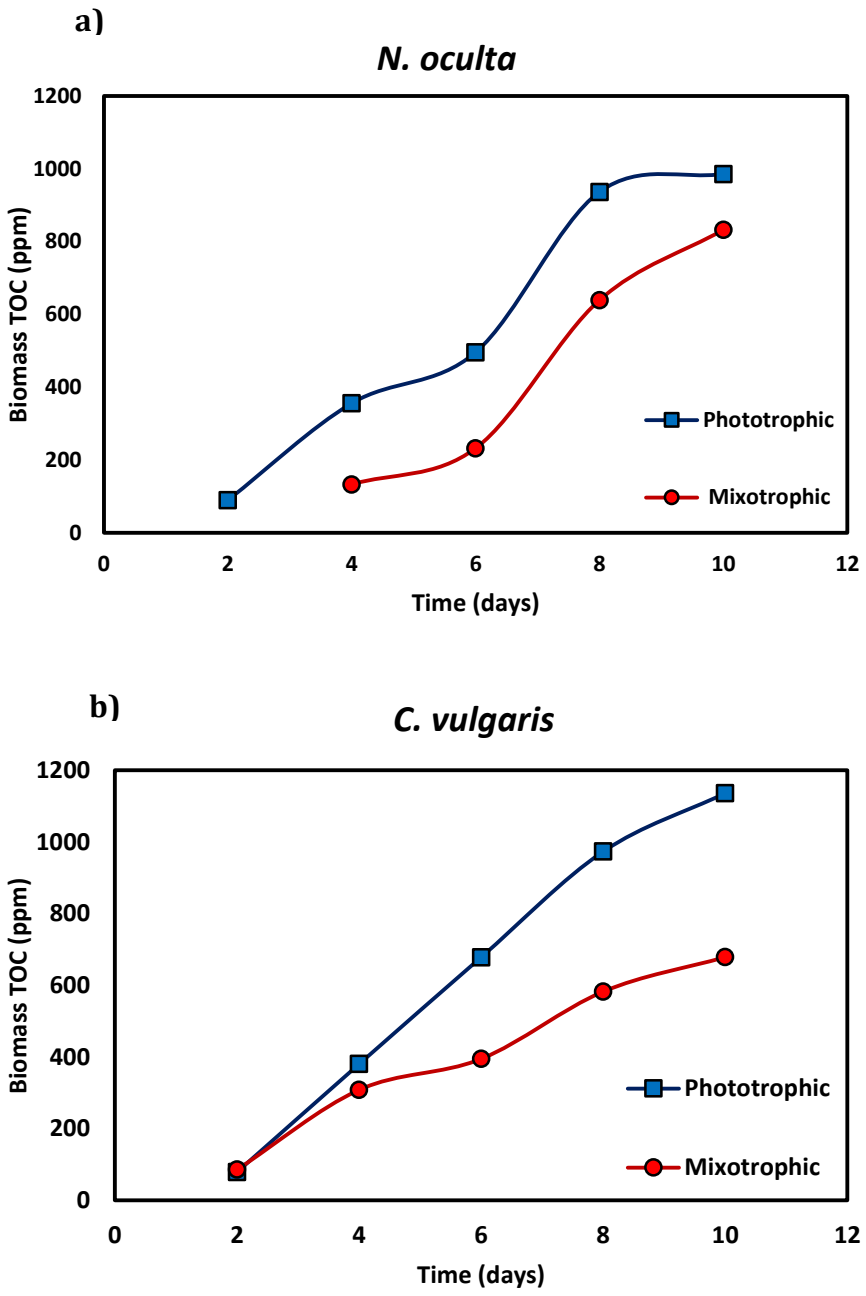


Figure 5-3 Accumulation of TOC in Microalgal Biomass a) *N. oculata*, b) *C. vulgaris*.

The Carbon content of microalgal biomass calculated as;

$$C_{total} = \frac{TOC_{Biomass}}{X} \times 100 \quad (5\text{-}1)$$

Where, $TOC_{Biomass}$ is the total organic carbon concentration of microalgal biomass (g L^{-1}), and X is the biomass concentration (g L^{-1}) at a given day. The

$TOC_{Biomass}$ is determined as follow;

$$TOC_{Biomass} = TOC_{Medium+Biomass} - TOC_{Filtered\ Medium} \quad (5\text{-}2)$$

Where $TOC_{Medium+Biomass}$ is the total organic carbon of the culture medium containing the microalgae, while $TOC_{Filtered\ Medium}$ is the total organic carbon of the filtered medium (free of microalgae).

Under phototrophic condition $TOC_{Filtered\ Medium} = 0$, hence the medium is free of organic carbon. While under mixotrophic condition $TOC_{Filtered\ Medium} \neq 0$, because the medium contains glucose, which is an organic compound.

The trend of the carbon content curve was found to be increasing with culturing duration. Moreover, the carbon content of cells cultured under phototrophic condition is higher than when cultivation conducted in mixotrophic condition. As we clarify earlier, that the cells like inorganic source of carbon (CO_2) more than the organic source (glucose). Figure 5-4a&b shows the changing level of carbon content for *N. oculata* and *C. vulgaris* respectively.

For the phototrophic mode, it's quite understood that all the carbon accumulated is captured from CO_2, which is an inorganic carbon source - if we neglect the initial cell presents in the medium before starting culturing. On the other hand, for

mixotrophic mode the cells use two sources of carbon as food, which are CO_2 and glucose. Figure 5-5 describes the percentage carbon content of microalgae species by the end of cultivation period.

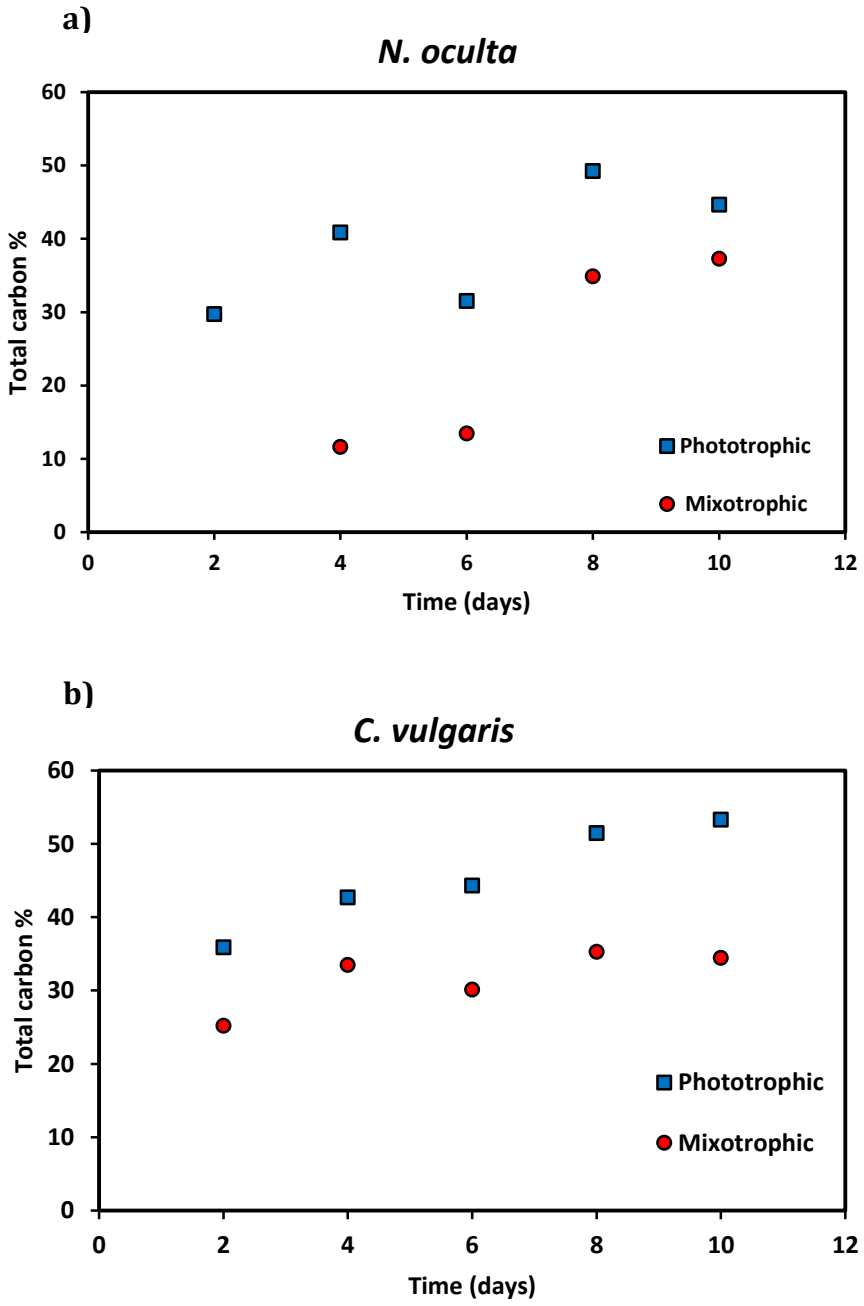
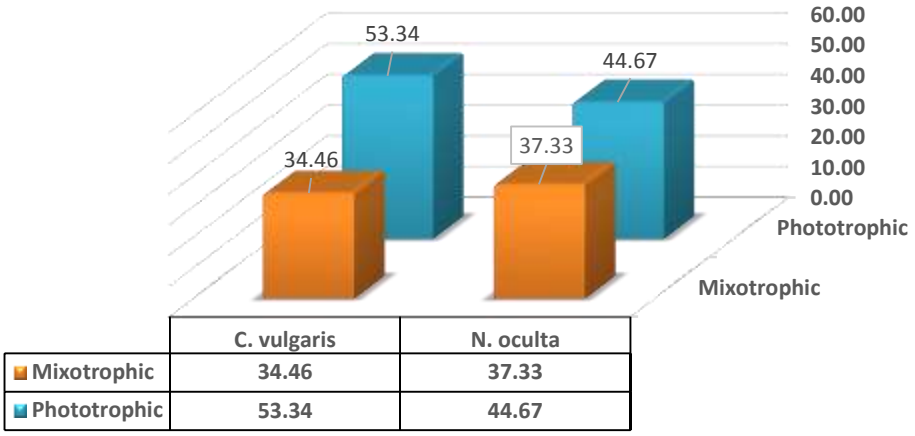


Figure 5-4 Carbon Content of Microalgae a) *N. oculata*, b) *C. vulgaris*.



	C. vulgaris	N. oculta
Mixotrophic	34.46	37.33
Phototrophic	53.34	44.67

Figure 5-5 The Final Carbon Content (%) of Microalgae Species Cultured under Phototrophic and Mixotrophic Conditions

5.4. TOC Uptake from the Mixotrophic Culture Medium

In this part, we want to analyze the changes in the culture medium TOC. In fact, in the phototrophic condition, there are no organic compounds present in the culture medium. Therefore, the following results are related to cultivation under mixotrophic condition only, since in this case organic source was added to the medium, which is glucose.

The glucose was removed from medium as the cells grow (bacteria action is also included). Hence, initially the TOC of the medium free of microalgae cells was 343 ppm approximately. However, by the end of culturing this number is reduced to a constant value of around 132 ppm and 117 for *N. oculata* and *C. vulgaris* respectively as described in Figure 5-6a&b.

A general notice is that TOC was not consumed totally, this might be due to depletion of other nutrients such as nitrogen and phosphorus. When other nutrient

totally consumed, the cells will stop growing, whatever the level of the organic source presents in the medium.

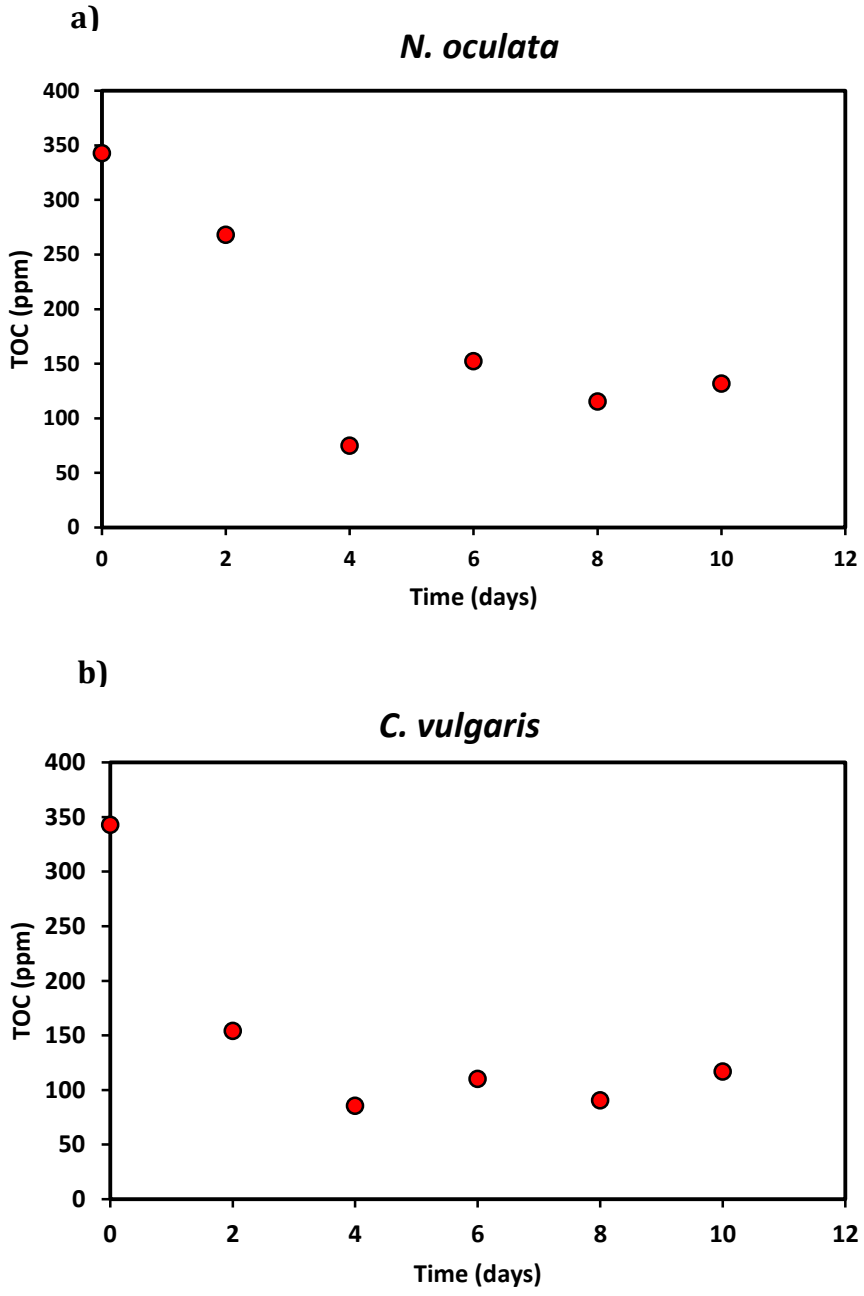


Figure 5-6 Depletion of TOC from the Mixotrophic Culture Medium for a) *N. oculata*, b) *C. vulgaris*.

5.5. Rate of CO2 Capture (Phototrophic Condition)

The rate of CO_2 capture can be estimated by the following equation [66], [100].

$$R_{CO_2} = PC_{CO_2} \frac{M_{CO_2}}{M_C} \tag{5-3}$$

Where R_{CO_2} is the rate of CO_2 fixation (g L^{-1} day^{-1}), P is the biomass productivity (g L^{-1} day^{-1}), M_{CO_2} is the molecular weight of carbon dioxide, and M_C is the molecular weight of carbon. While C_{CO_2} is the carbon content of microalgae biomass obtained only from CO_2.

The biomass productivity determined using the following equation.

$$P = \frac{X_i - X_o}{t_i - t_o} \tag{5-4}$$

Where, X_i is the biomass concentration (g L^{-1}) at time t_i (days), X_o is the initial biomass concentration (g L^{-1}) at time t_o (days).

The CO_2 bio-fixation rate is determined based on the carbon content of microalgae cells obtained from just CO_2. Hence, the initial load of cells carbon was ignored because the culturing was started with very low biomass concentration of around 0.015 g/L. In phototrophic cultivation C_{CO_2} is the same as C_{total}. While in mixotrophic cultivation, C_{CO_2} cannot be calculated because there are two sources of carbon for microalgae to grow, which are CO_2 and glucose.

However, the CO_2 consumption rate was estimated by multiplying the biomass productivity with the carbon content equivalent CO_2. The biomass productivity determined as the change in biomass concentration over time. Therefore, CO_2 capturing rate increase by increasing the biomass productivity or the carbon content of the biomass obtained from CO_2. For real municipal wastewater, it anticipated that biomass productivity would be in lower side compared to the growth in BBM medium rich of nutrients. As a result, the CO_2 bio-fixation rate will be lower than the values obtained in this study.

Table 5-1. Lists the growth parameters and the daily CO_2 bio-fixation rate of *N. oculata* and *C. vulgaris*. The reported maximum CO_2 capturing rate under phototrophic were 0.43 g/L/day and 0.44 g/L/day for *N. oculata* and *C. vulgaris* respectively.

Table 5-1 Growth Kinetics and Rate of CO2 Capture under Phototrophic Condition

Days	*N. oculata* – Phototrophic				*C. vulgaris* – Phototrophic			
	X (g/L)	*(g/L/day)*	$\%C_{CO_2}$	CO_2 captured *(g/L/day)*	*X (g/L)*	*P (g/L/day)*	$\%C_{CO_2}$	CO_2 captured *(g/L/day)*
0	0.015	0	-	-	0.015	0	-	-
2	0.3	0.14	29.77	0.16	0.22	0.10	35.92	0.14
4	0.87	0.21	40.87	0.32	0.89	0.22	42.73	0.34
6	1.57	0.26	31.57	0.30	1.53	0.25	44.34	0.41
8	1.9	0.24	49.29	0.43	1.89	0.23	51.50	0.44
10	2.21	0.22	44.67	0.36	2.13	0.21	53.34	0.41

Additional plot (Figure 5-7) is provided to analyze the general trend of microalgae species towards CO_2 capturing. It can be clearly seen that the daily CO_2 consumption rate goes up with culture duration. This is mainly due to the increment in C_{CO_2}. Since the productivity does not change so much except in the early days, wherein its value rose rapidly, but after that it keeps fluctuating in a narrow range.

Tang et al. [66] studied the carbon content and the rate of CO_2 capture for different microalgae strains cultivated in modified BG11 medium, by using a range of CO_2 feed concentration starting from 0.03% up to 50 %. The carbon content was again around 50 % of the total biomass, while the maximum rate of CO_2 bio-fixation was between 0.105-0.288 g L^{-1} day^{-1}.

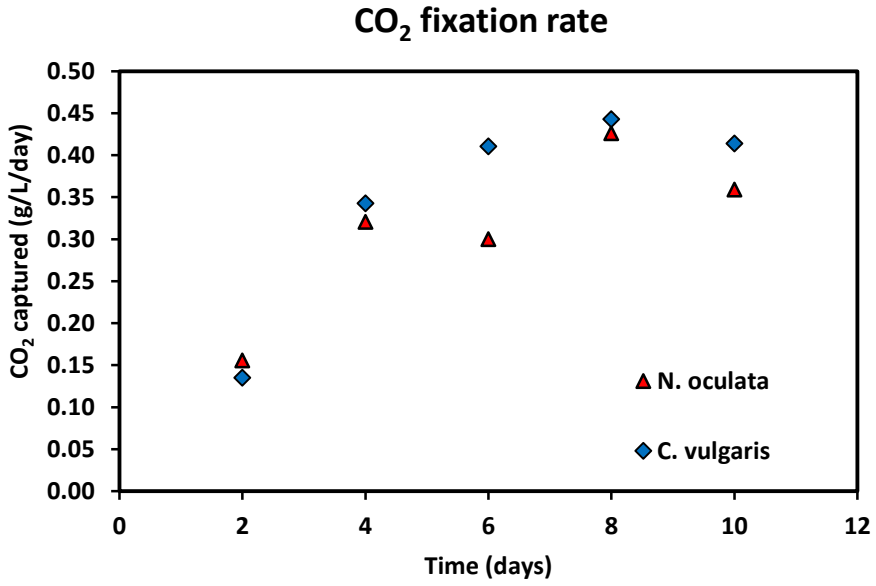


Figure 5-7 Rate Of CO_2 Capture under Phototrophic Condition for *N. oculata* and *C. vulgaris*.

5.6. Conclusion

- Culturing of microalgae species under phototrophic condition gives higher cells carbon content and rate of CO_2 capture than when mixotrophic cultivation is implemented.
- Microalgae cells prefer the inorganic source of carbon (CO_2) than the organic carbon source (glucose).
- Integration of a microalgae culturing unit within the wastewater plant should be on the tertiary stage. Since, in this case the effluent from secondary treatment units that does not contain organic carbon allow the phototrophic culturing of microalgae, which will insure high CO_2 capturing rate.

CHAPTER 6

NUTRIENTS UPTAKE AND REMOVAL

Microalgae can be effectively utilized to remove inorganic nutrients (nitrogen and phosphorus compounds) from municipal wastewater in the tertiary treatment phase, hence the domestic wastewater includes substantial amounts of these nutrients for the algae growth [24]. In fact, the secondary wastewater effluent contains typically low concentrations of nitrogen and phosphorus, microalgae can achieve high removal percentage of these inorganic nutrients [58].

In this study, we investigate nutrients uptake and removal from wastewater effluent from the secondary treatment. Variable initial nitrogen and phosphorus concentrations were considered, due to the variations in the upcoming wastewater characteristics as a result of daily and seasonal fluctuations.

6.1. Nitrogen Uptakes and Removal

The synthetic wastewater was prepared by addition of ammonium chloride (NH_4Cl) as the only source of nitrogen. However, to study the effect of nitrogen removal, different initial TN-Ammonia were adjusted to be within the range of 20 to 75 mg/L, and the concentration of phosphorus compounds TP- Phosphate was kept constant at 9.0 ± 0.1 mg/L and 10.8 ± 0.3 mg/L for *N. oculata* and *C. vulgaris* respectively, for all TN-Ammonia initial values.

The metabolic consumption of ammonium NH_4^+ as a nitrogen source were described by the following reaction [101], [102], hence firstly ammonium dissociated into ammonia (NH_3) and hydrogen ion (H_+). Ammonia is consumed by microalgae cells, while the produced hydrogen ions in the aqueous media might leads to reduction in pH of the culture media [102].

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \rightleftharpoons Org - N + H^+$$

Additionally, ammonium (NH_4^+) might be removed from culture media by ammonia (NH_3) desorption method. Thus, the following equilibrium reaction shows how ammonium is converted into ammonia, and this occurs when the pH is greater than 7 which increase the formation of ammonia. Again, this reaction will result in lowering the culture media pH, since OH^- ions were consumed in the reaction. Accordingly, ammonia gas gets stripped by the air stream, hence mixing and gas flow rate could have impacts on nitrogen removal [102].

$$NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O$$

In the present study, we consider the total elimination of ammonia, whether by ammonia stripping or by cells' consumption. Figure 6-1a&b shows TN-Ammonia uptake from culture media using *N. oculata* and *C. vulgaris*. In addition, Figure 6-2a&b shows the percentage removal of TN-Ammonia from synthetic wastewater by culturing *N. oculata* and *C. vulgaris*.

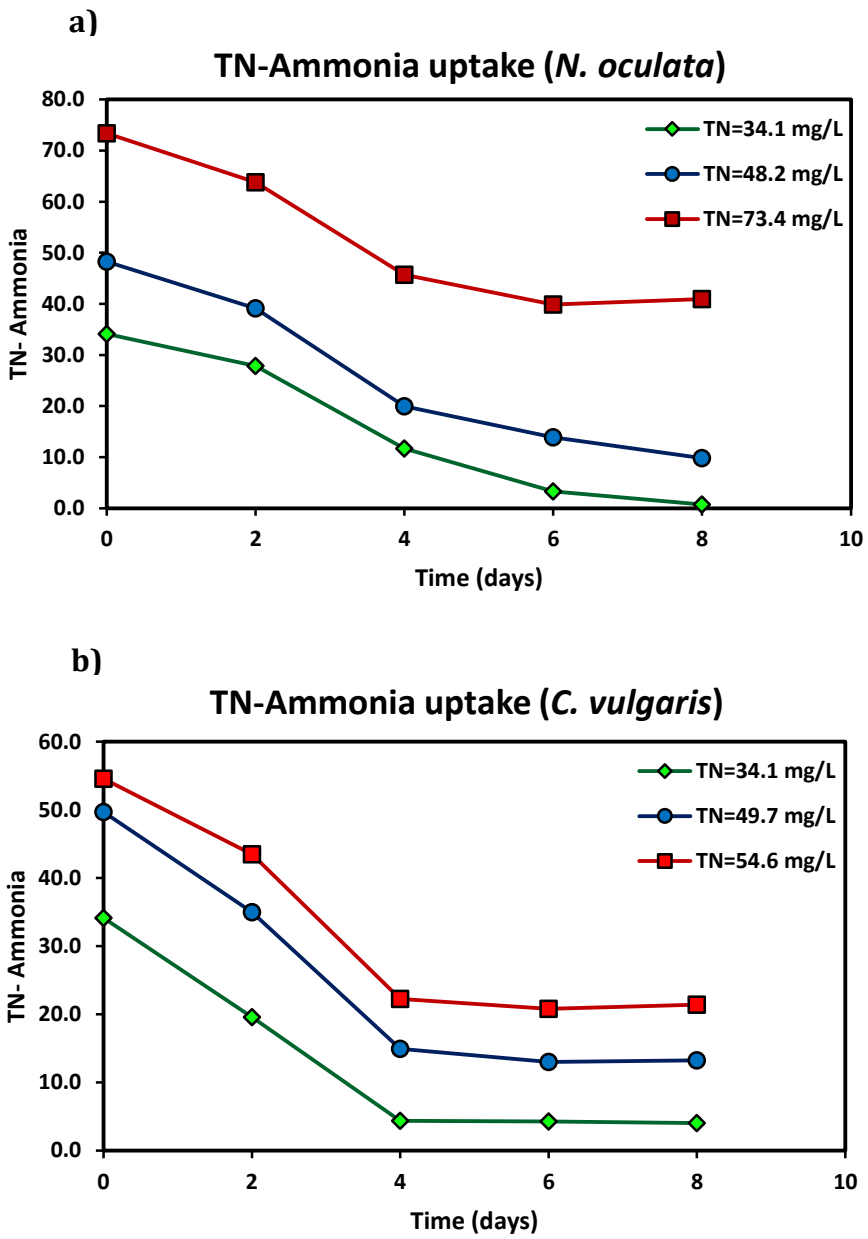


Figure 6-1 TN-Ammonia Uptake from Synthetic Wastewater Media Using a) *N. oculata*, b) *C. vulgaris*.

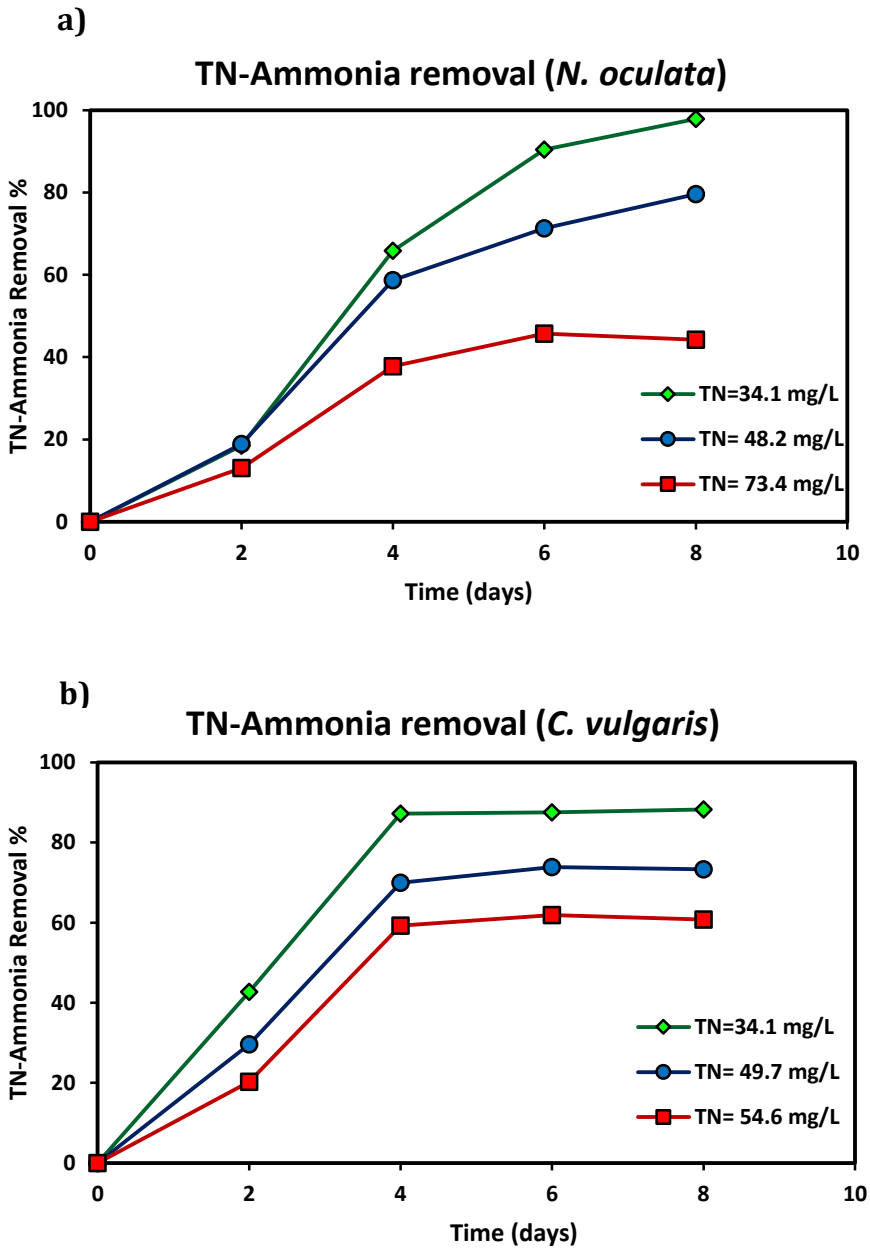


Figure 6-2 TN-Ammonia Removal from Synthetic Wastewater Media Using a) *N. oculata*, b) *C. vulgaris*.

As was observed from the Figures 6-1a&b for *N. oculata* and *C. vulgaris*, the nutrients uptake at different initial TN-Ammonia has shown similar trends for each microalgae species. However, the nitrogen level decline gradually during cultivation period. Finally, the TN-Ammonia concentration approach a constant value after the cultivation duration of almost 6 and 4 days for *N. oculata* and *C. vulgaris* respectively. However, at this point, other nutrients are already consumed, therefore cells growth will stop spontaneously. In other words, both nitrogen and phosphorus component must exist for cells growth, when one source is totally consumed the growth will definitely stop whatever the concentration of other components.

The results were further confirmed by analyzing the trend of TN-Ammonia removal curves described in Figure 6-2. It can be clearly seen that nitrogen was removed faster using *C. vulgaris* than the case when *N. oculata* is used. Moreover, the degree of removal is also depends on the total nitrogen concentration present initially in culture media.

6.2. Phosphorus Uptake and Removal

Phosphorus is also an important component for energy metabolism of microalgae, and it compose some biomass constituents such as acids, lipids, proteins [41]. For phosphorous removal investigation, TP- Phosphate were maintained between 3 to 16 mg/L, and TN-Ammonia was kept constant at 46.0 $\pm$ 1.4 mg/L and 49.9 $\pm$ 1.5 mg/L for *N. oculata* and *C. vulgaris* respectively, for all TP-Phosphate initial values.

In facts, phosphorus is involved in nucleic-acid synthesis, specifically for energy transfer processes. In addition, the uptake of phosphorus depends on many factors, including its concentration in the media, pH, and the concentration of other cation micronutrients (Na^{+}, K^{+} and Mg^{2+}). However, microalgae cells utilize phosphorus compounds in the formation of organic and inorganic constituents through three different processes. These processes are phosphorylation, oxidative phosphorylation, and photo-phosphorylation. [103]. In the photophosphorylation process, phosphorus is required for ATP (adenosine triphosphate) synthesis. Hence the existence of phosphorus compounds in culture media has a significant impact on the photosynthesis process and cells growth [69].

Figure 6-3a&b shows TP-Phosphate uptake from culture media using *N. oculata* and *C. vulgaris*. In addition, Figure 6-4a&b shows the percentage removal of TP-Phosphate from synthetic wastewater media by using *N. oculata* and *C. vulgaris*.

A general notice is that TP-Phosphate was consumed quickly by microalgae cells of both strains, as clear from Figure 6-3a&b. However, by day 4, phosphorus compound was totally consumed. This because in synthetic wastewater the phosphorus concentration is very small in comparison with nitrogen concentration. Therefore, cells will keep growing unless the phosphorus is consumed completely. The same justification is valid for TP-Phosphate removal plots shown in Figure 6-4a&b. Since, the removal curves went up sharply to 100% removal after a few days, and for all levels of initial total phosphorus concentration.

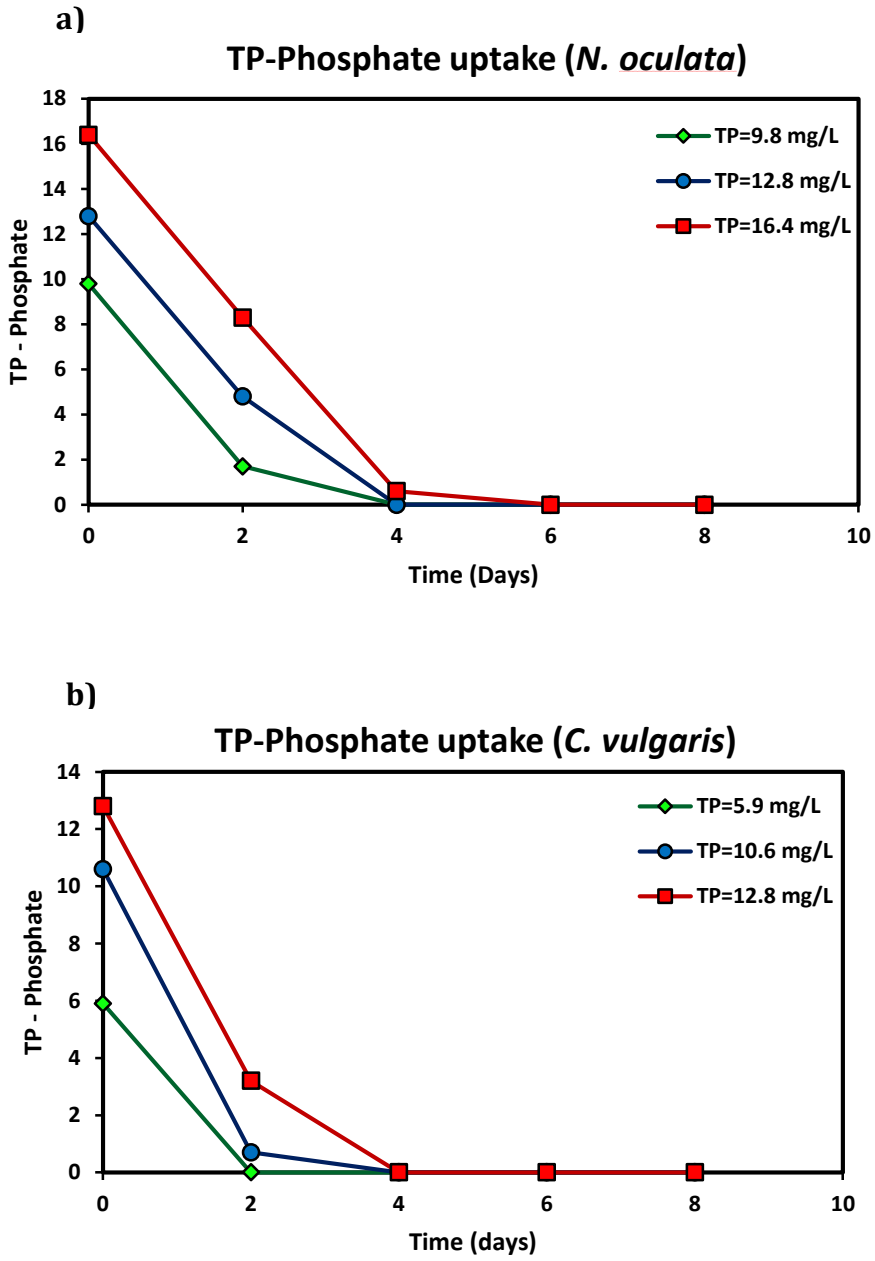


Figure 6-3 TP-Phosphate Uptake from Synthetic Wastewater Media Using a) *N. oculata*, b) *C. vulgaris*.

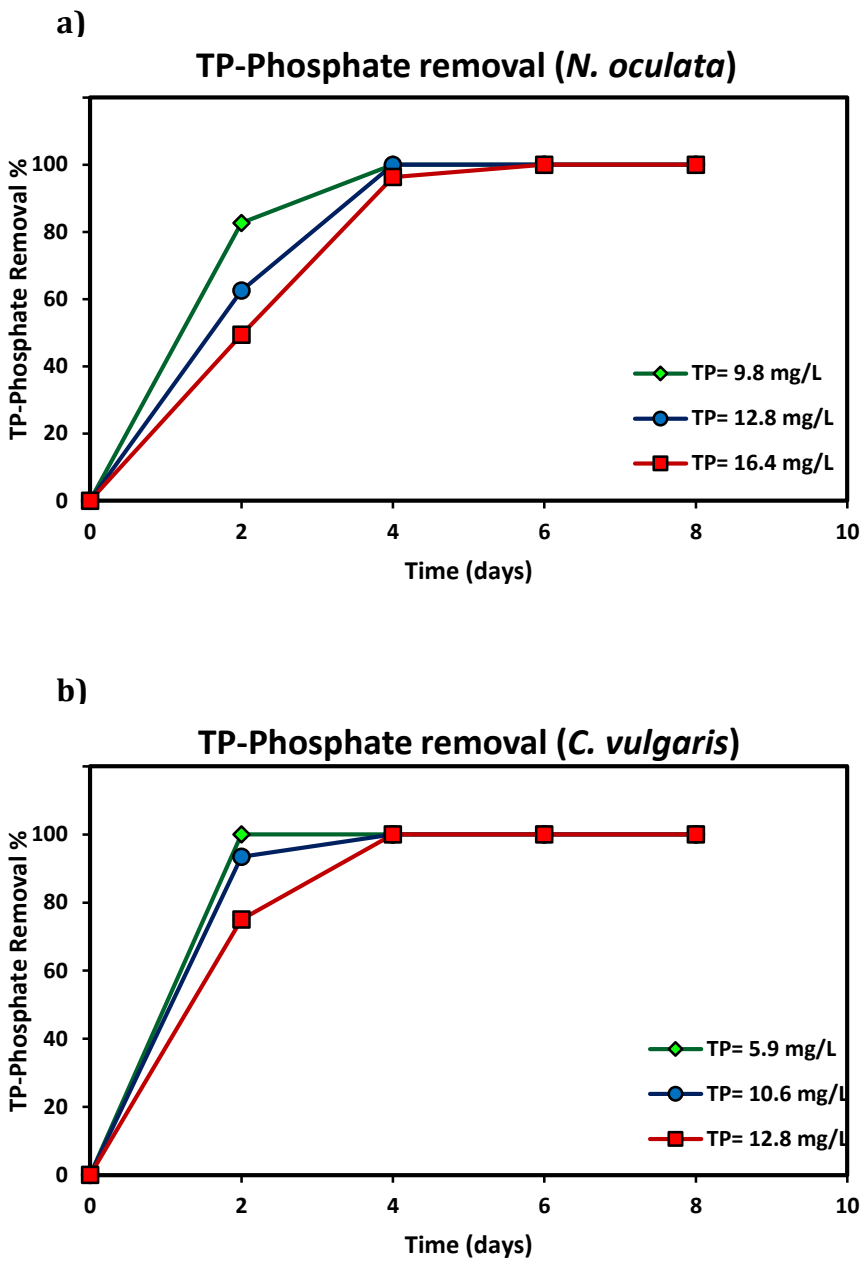


Figure 6-4 TP-Phosphate Removal from Synthetic Wastewater Media Using a) *N. oculata*, b) *C. vulgaris*.

6.3. Effects of N/P Ratio

In fact, both nitrogen and phosphorus could be removed efficiently if the N/p ratio in the wastewater media in a proper range [58]. However, the high N/P ratio will reduce the nitrogen removal rate. Since, in this case the phosphorus concentration is less in comparison with nitrogen concentration. Accordingly, when phosphorus compounds depleted from the media, the microalgae cell will stop growing and uptaking nitrogen. As consequences, most of the nitrogen compounds will not be removed and will remain in culture media. As a result, a low removal percentage of nitrogen occurs when we have a high N/P ratio. Table 6-1 and 6-2 lists nitrogen and phosphorus percentage removal at different N/P values for both microalgae strains.

Table 6-1 Effects of N/P Ratio on TN and TP Removal for *N.oculata*.

	Variation in nitrogen concentration		
TN-Ammonia (mg/L)	34.14	48.24	73.39
TP- Phosphate (mg/L)	9.00	9.00	8.80
N/P	3.79	5.36	8.34
TN Removal (%)	97.86	79.60	44.21
	Variation in phosphorus concentration		
TN-Ammonia (mg/L)	39.50	37.80	37.40
TP- Phosphate (mg/L)	16.40	12.80	9.80
N/P	2.41	2.95	3.82
TP Removal (%)	100	100	100

Table 6-2 Effects of N/P Ratio on TN and TP Removal for *C. vulgaris*.

	Variation in nitrogen concentration		
TN-Ammonia (mg/L)	34.14	49.69	54.55
TP- Phosphate (mg/L)	10.50	10.70	10.80
N/P	3.25	4.64	5.05
TN Removal (%)	88.26	73.35	60.80
	Variation in phosphorus concentration		
TN-Ammonia (mg/L)	39.30	41.90	41.60
TP- Phosphate (mg/L)	12.80	10.60	5.90
N/P	3.07	3.95	7.05
TP Removal (%)	100	100	100

6.4. Conclusion

- The results show that phosphorus compounds consumed easier than nitrogen compounds due to the tiny initial concentration of phosphorus compounds.
- For *N. oculata* TN-Ammonia percentage removal on day 8 were 97.9%, 79.6%, 44.2% for initial TN-Ammonia concentration of 34.1, 48.2, 73.4 mg/L respectively.
- While, for *C. vulgaris* TN-Ammonia percentage removal were 88.3%, 73.3%, 60.8% for initial TN-Ammonia concentration of 34.1, 49.7, 54.6 mg/L respectively.
- For both microalgae species TP-Phosphate removal reached quickly to 100% on day 6 for different initial TP-Phosphate concentration.

CHAPTER 7

BIOMASS THERMAL CONVERSION

This study was aimed to investigate the high temperature decomposition behavior of microalgal biomass for *N. oculata* and *C. vulgaris*, different approaches were used to evaluate the reaction kinetics including direct model fitting technique, and model-free methods.

7.1. Kinetic Modeling

The high temperature non-isothermal degradation of biomass is a function of temperature and the fractional amount of the remnant solid. Thus, the reaction rate can be described by the following equation.

$$\frac{d\alpha}{dt} = k(T) f(\alpha) \tag{7-1}$$

Where, t is the reaction time (in min); T is absolute temperature (in K); α is the fractional conversion and is defined as;

$$\alpha = 1 - W_f \tag{7-2}$$

Since W_f represents the remnant weight fraction of the sample. $k(T)$ is the reaction rate constant which is function of temperature. Using the Arrhenius expression for the reaction rate constant, the overall rate of reaction becomes,

$$\frac{d\alpha}{dt} = k_o \exp\left(-\frac{E_a}{RT}\right) f(\alpha) \tag{7-3}$$

where, R is universal gas constant (in kJ/mol K); E_a is the apparent activation energy (in kJ/mol); k_o is the frequency factor (in 1/min); and n is the overall order of degradation.

The apparent activation energy (E_a) can be obtained by two approaches. The first approach required to specify $f(\alpha)$ depending on the proposed reaction mechanism, and by direct fitting procedure E_a and other reaction kinetic parameters can be estimated. While the second approach is independent of the reaction mechanism and it is called model-free methods.

7.1.1. Direct model fitting

We assume $f(\alpha)$ as the nth order reaction rate model, then the overall rate of degradation can be expressed as follows, [104]–[107]

$$\frac{d\alpha}{dt} = k_o \exp\left(-\frac{E_a}{RT}\right)(1-\alpha)^n \qquad (7\text{-}4)$$

The kinetics parameters of Eq. (7-4) which are E_a, k_o and n, were evaluated by fitting Eq. (7-4) to the experimental data. FindFit and NonlinearModelFit commands in Mathemaica 9 software were used to evaluate the unknown parameters for each heating rate. Maximum 1000 iterations was used to achieve a specified precision, since that the fitting commands generate many solutions and the selection was based on the highest coefficient of determination R^2. The coefficient of determination was estimated using "RSquared" command.

In order to compare between the thermogravimetric (TG) profile (α versus T) of the model and the experiments, we need a relationship between α and T to obtain

the TG curve for the model after substitution of the estimated parameters. Therefore, we must firstly expressing Eq. (7-4) to be as a function of temperature only and then solving it. This was done by replacing the time differential according to the given relation between temperature, time and the heating rate ($\beta = dT/dt$), where β is the heating rate (^{o}C/min). Thus, Eq. (7-4) becomes

$$\frac{d\alpha}{dT} = \frac{k_o}{\beta}\exp\left(-\frac{E_a}{RT}\right)(1-\alpha)^n \qquad (7\text{-}5)$$

Eq. (7-5) can be solved by two procedure, either by the approximate analytical solution or by the numerical solution.

Approximate analytical solution

Firstly, Eq. (7-5) was arranged, followed by taking the integral of the both side of the equation

$$\int_0^{\alpha}\frac{d\alpha}{(1-\alpha)^n} = \frac{k_o}{\beta}\int_{T_o}^{T} exp\left(-\frac{E_a}{RT}\right)dT \qquad (7\text{-}6)$$

Where, T_o refers to the initial experiment temperature (in K). And the left hand sight of was defined as $g(\alpha)$.

$$g(\alpha) = \int_0^{\alpha}\frac{d\alpha}{(1-\alpha)^n} = \frac{1-(1-\alpha)^{1-n}}{(1-n)} \qquad (7\text{-}7)$$

Although, the right hand side of Eq. (7-6) has no analytical solution [108], the Coats-Redfern approximation could be used [109].

Given that the value of $E_a/RT \gg 1$, then the right hand side of Eq. (7-6) could be approximated as follow [110].

$$\int_{T_o}^{T} exp\left(-\frac{E_a}{RT}\right)dT \approx \frac{R}{E_a}T^2 exp\left(-\frac{E_a}{RT}\right) \qquad (7\text{-}8)$$

Now, after substitution of Eq. (7-7) and Eq. (7-8) in Eq. (7-6), and by taking the natural logarithmic for both sides we get

$$ln\left[\frac{1-(1-\alpha)^{1-n}}{(1-n)T^2}\right] = ln\left[\frac{k_o R}{\beta E_a}\right] - \frac{E_a}{RT} \qquad (7\text{-}9)$$

The same results of Eq. (7-9) is obtained by [111], further rearrangement of Eq. (7-9) yields;

$$W_f = (1-\alpha) = \left[1-(1-n)T^2\frac{k_o R}{\beta E_a}exp\left(-\frac{E_a}{RT}\right)\right]^{\frac{1}{1-n}} \qquad (7\text{-}10)$$

Then, TG curve from model was plotted after substituting the estimated parameters values in Eq. (7-10).

For thermogravimetric (TG) and differential thermogravimetric (DTG) profiles modeling, data were selected from experimental results starting from temperature of 30 ^{o}C and with increment of 10 ^{o}C the total number of point selected were in the range of (65 – 78 points), for plotting of the experimental data for Figure 2 and Figure 3 half of the points are chosen in order to get a clear and neat graphs.

Numerical solution

Numerical solution for α as a function of temperature was obtained by solving Eq. (7-5) using NDSolve command in Mathemaica 9 software. The boundary condition is α [298 K] = 0, which at the initial experimental temperature (normally 298 K) the sample weight loss not started.

7.1.2. Model-free methods

These methods are used to determine the unknown apparent activation energy (E_a) without specifying a model to describe the reaction mechanism, there is no need to define $f(\alpha)$ or $g(\alpha)$. the following two iso-conversional methods are commonly used [112]:

Kissinger–Akahira-Sunose (KAS) method

This methods is briefly described as [113], [114].

$$\ln\left[\frac{\beta}{T^2}\right] = ln\left[\frac{k_o R}{E_a g(\alpha)}\right] - \frac{E_a}{RT} \qquad (7\text{-}11)$$

According to this method, the plots of $\ln\left[\frac{\beta}{T^2}\right]$ versus $1/T$ for different α values is a straight line and the E_a can be calculated from the slope of the line. Though, there is an E_a value at each fractional conversion α.

Flynn-Wall-Ozawa (FWO) method

The alternative linearized form of the model equation [113], [114]

$$\ln[\beta] = ln\left[\frac{0.0048 k_o E_a}{R\, g(\alpha)}\right] - 1.0516\frac{E_a}{RT} \qquad (7\text{-}12)$$

Again, $\ln[\beta]$ *versus* $1/T$ gives straight line. The E_a can be calculated from the slope of the line. Also, there is an E_a value at each fractional conversion α.

7.2. Thermal Characteristics of Microalgae Strains

Table 7-1 shows the approximate and ultimate analysis of *N. oculata* and *C. vulgaris*. Approximate analysis is found that the moisture content, ash, volatile matter, fixed carbon and heating values are different for *N. oculata* than those of *C. vulgaris* in the culture product. It can be even more confirmed that the elemental carbon, hydrogen and oxygen content also higher for *N. oculata*.

TG and DTG profiles show in Figure 7-1 for *N. oculata* and *C. vulgaris* at different heating rates 5, 10, 15 and 20 ^{o}C /min. One can see both the microalgae species shows similar thermal behavior in the first burning stage up to temperature of 220 °C. In case of *C. vulgaris* a small change in trend was observed just before the maximum peak while this change not recorded clearly for *N. oculata*. Also, *N. oculata* shows high decomposition rate at the last peaks than *C. vulgaris* confirmed from DTG plots for both strains.

In general, the thermal decomposition processes can be classified into three stages, first stage where the devolatilization of volatile compounds and moisture occur, at the second sages most of the weight loss is observed, and the last stage wherein weight change slowly at high temperature near the end of decomposition process [104], [114]. However, at earliest stage there is slight weight change at temperature around 100 °C which is mainly due to moisture evaporation. The second decomposition stage start from 200 °C to 400 °C roughly, though this weight loss is a result of combustion of proteins and carbohydrates compounds [115], other small peaks appears till a temperature of 650 °C approximately. Finally at

temperature higher than 650 °C, the weight loss decreases slowly and this stage is associated wish decomposition of the solid residual [116].

Moreover, the maximum peak temperature (T_{peak}) for *N. oculata* is slightly higher than *C. vulgaris*for all heating rates. Also, the corresponding maximum decomposition rates $(d\alpha/dt)_{max}$ and $(d\alpha/dT)_{max}$ is higher in *N. oculata* than *C. vulgaris* as shown in Table 7-2. If these temperatures at the maximum peak temperature (T_{peak}) for microalgae biomass decomposition compared to other biomass like corncob, sawdust, palm shell and coconut shell as described by [117], we found that *N. oculata* and *C. vulgaris* have the lowest temperatures.

Ash is considered as byproduct for biomass thermal decomposition, however in real operation like in pyrolysis and gasification high values of ash content is undesirable and its would influence the process design and operation since it decreases the final products quality and consequently the need for purification process [118]. Ashes in microalgae mainly formed by metal compounds, however different pre-treatment processes such as washing before the combustion processes are suggested by several researches to reduce the mineral content [94]. In this study, *C. vulgaris* showed slightly high ash content than *N. oculata* and in term of heating value is better *N. oculata*, as recorded in Table 7-1.

Table 7-1 Proximate Analysis and Ultimate Analysis

	N. oculata	*C. vulgaris*
Proximate analysis (%)		
Moisture	6.71 ± 0.29	6.89 ± 0.44
Ash	6.40 ± 0.47	6.86 ± 1.41
Volatile matter	78.94 ± 2.61	78.40 ± 2.05
Fixed carbon	7.95	7.85
Heating value (MJ/kg)	15.07	14.94
Ultimate analysis (%)		
C	40.98	40.67
H	5.31	5.27
O	39.99	39.70

*The standard deviation calculated using STDEV in Microsoft Excel 2010.

*Fixed carbon calculated by difference.

Table 7-2 Maximum Peak Characteristics

	N. oculata			*C. vulgaris*		
β	T_{peak}	$\left(d\alpha/dt\right)_{max}$	$\left(d\alpha/dT\right)_{max}$	T_{peak}	$\left(d\alpha/dt\right)_{max}$	$\left(d\alpha/dT\right)_{max}$
5	278.20±0.35	3.42±0.01	0.683±0.003	277.38±0.82	3.31±0.01	0.662±0.001
10	283.47±0.16	6.78±0.04	0.678±0.004	282.61±0.42	6.49±0.12	0.649±0.012
15	285.50±0.02	10.82±0.21	0.722± 0.014	286.19±0.18	10.42±0.19	0.695±0.013
20	287.24±0.23	15.26±0.01	0.763±0.001	287.16±0.60	14.02±0.65	0.701±0.032

*The standard deviation calculated using STDEV in Microsoft Excel 2010.
* T in (^{o}C) , $d\alpha/dt$ in (wt%/ min) , $d\alpha/dT$ in (wt%/K).

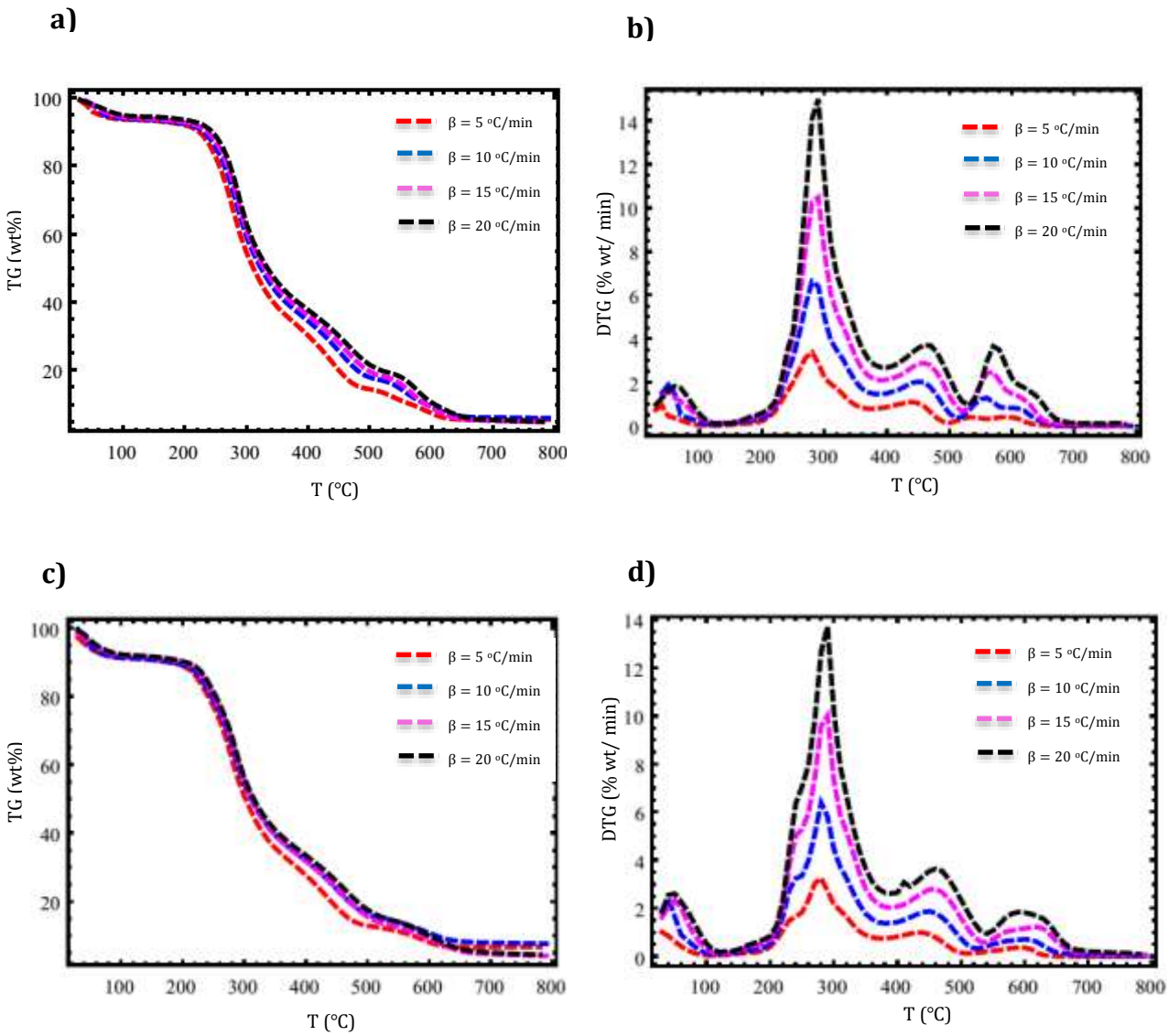


Figure 7-1 a) TG curve for *N. oculata* for different heating rate. b) DTG curve for *N. oculata* for different heating rate. c) TG curve for *C. vulgaris* for different heating rate. d) DTG curve for *C. vulgaris* for different heating rate

7.3. Effects of Heating Rate

Generally, when the heating rate increased the temperature at the maximum peak is tend to be delayed , a similar results was reported by Chen et al. [119] and [120] for *C. vulgaris* decomposition.

Gai et al. [121] postulate that since the biomass has poor thermal conductivity, so with increasing the heating rate the temperature gradient inside the biomass is increased resulting into limited heat transfer from the furnace pan to the sample, and therefore shifting the decomposition temperatures, on other words delay will occur in the sample temperature due to the rapid change in the furnace temperature. Also they argued that when heating rate is increased the residence time of the sample inside the combustion furnace is shorten and as consequences the corresponding initial and final decomposition temperatures are get affected (delayed). This evidences supported again by Liang et al. [122] for the thermal decomposition process of smooth cordgrass, hence they refer the shifting of the initial temperature for main decomposition stage, to the temperature gradient across the biomass when high heating rates is used. In addition, Idris et al. [123] reported that when heating rate is decreased, residual of the thermal decomposition reaction decreased too.

7.4. Evaluation of the Model-Fitting Method

Experimental and modeling TG curves at a) $\beta = 5°C/\min$. b) $\beta = 10°C/\min$, c) $\beta = 15°C/\min$. d) $\beta = 20°C/\min$ for *N. oculata* show in Figure 7-2 and for *C. vulgaris* in Figure 7-3. The fitting of the thermal weight loss with the n^{th} order rate model described by Eq. (7-4) shows excellent prediction to the experimental values of the decomposition processes for both species. However, model-fitting enables using of just one single heating rated each time for determination of reaction kinetics parameters including the activation energy, and actually this is considered as drawback, since the activation energy is get affected by the heating rate due to mass and heat transfer consequences [110].

Table 7-3 shows the estimated reaction kinetic parameters using the n^{th} order rate model described by Eq. (7-4). Although, the proposed model shows best fitting the experimental data but the coefficient of determination (R^2) values from Table 7-3 are to some extent much lesser than unity. This because for such nonlinear model is misleading to use the coefficient of determination R^2. Therefore, the coefficient of determination R^2 is an inappropriate measure for the goodness of fit in case of nonlinear models [124]. More discussions about fitting data to nonlinear models, R^2 determination and its suitability, and about nonlinear methods for regression are provided by [124]–[126]. Other statistical expression to examine the fitting accuracy for nonlinear modeling of the thermal decomposition of biomass is used by Vamvuka et al. [127] which is based on the deviation between the observed and calculated $(d\alpha/dt)$ with respect to the maximum observed $(d\alpha/dt)$.

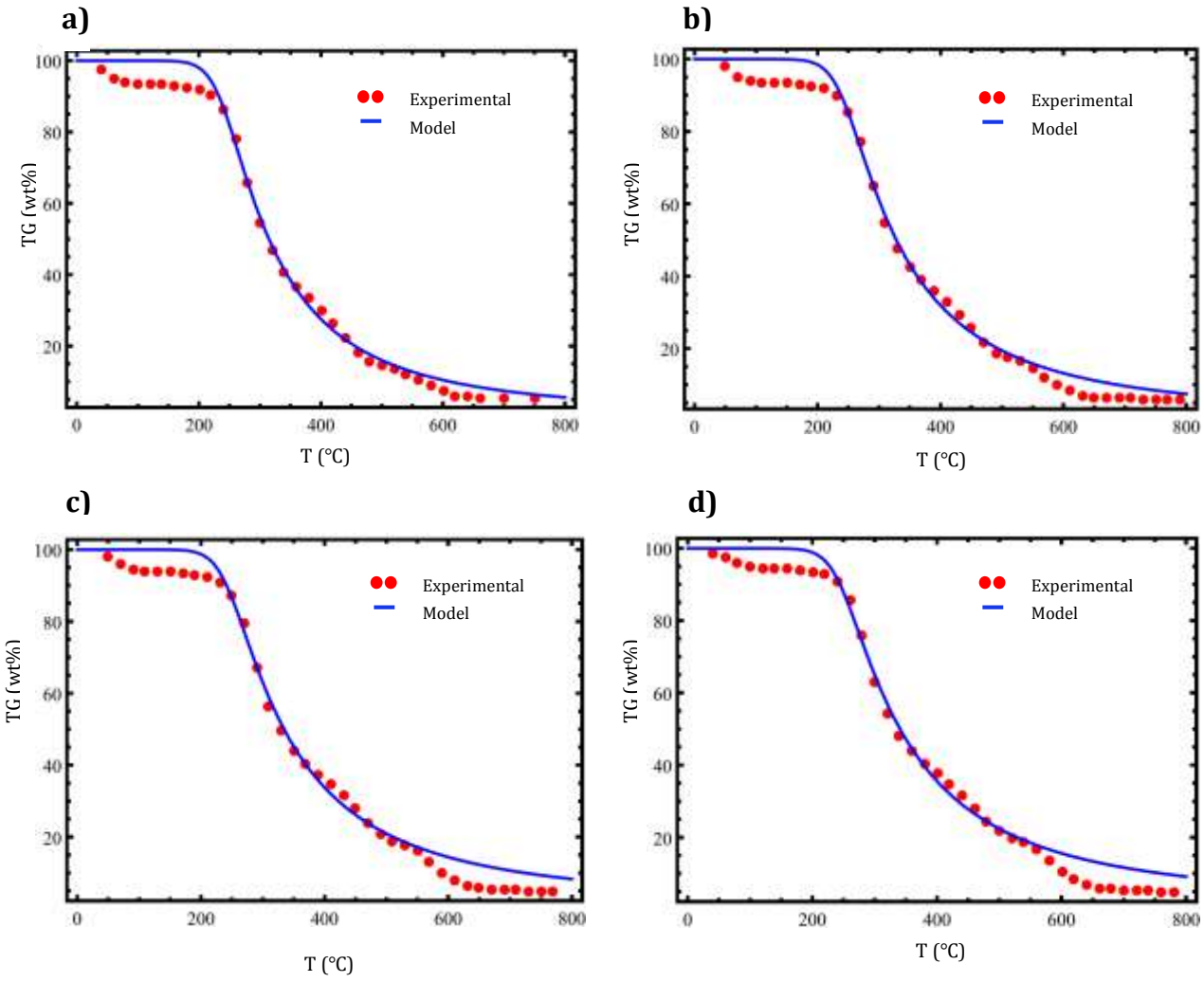


Figure 7-2 Experimental and Model TG Curves for *N. oculata* at a) β=5°C/min. b) β=10°C/min, c) β=15°C/min. d) β=20°C/min.

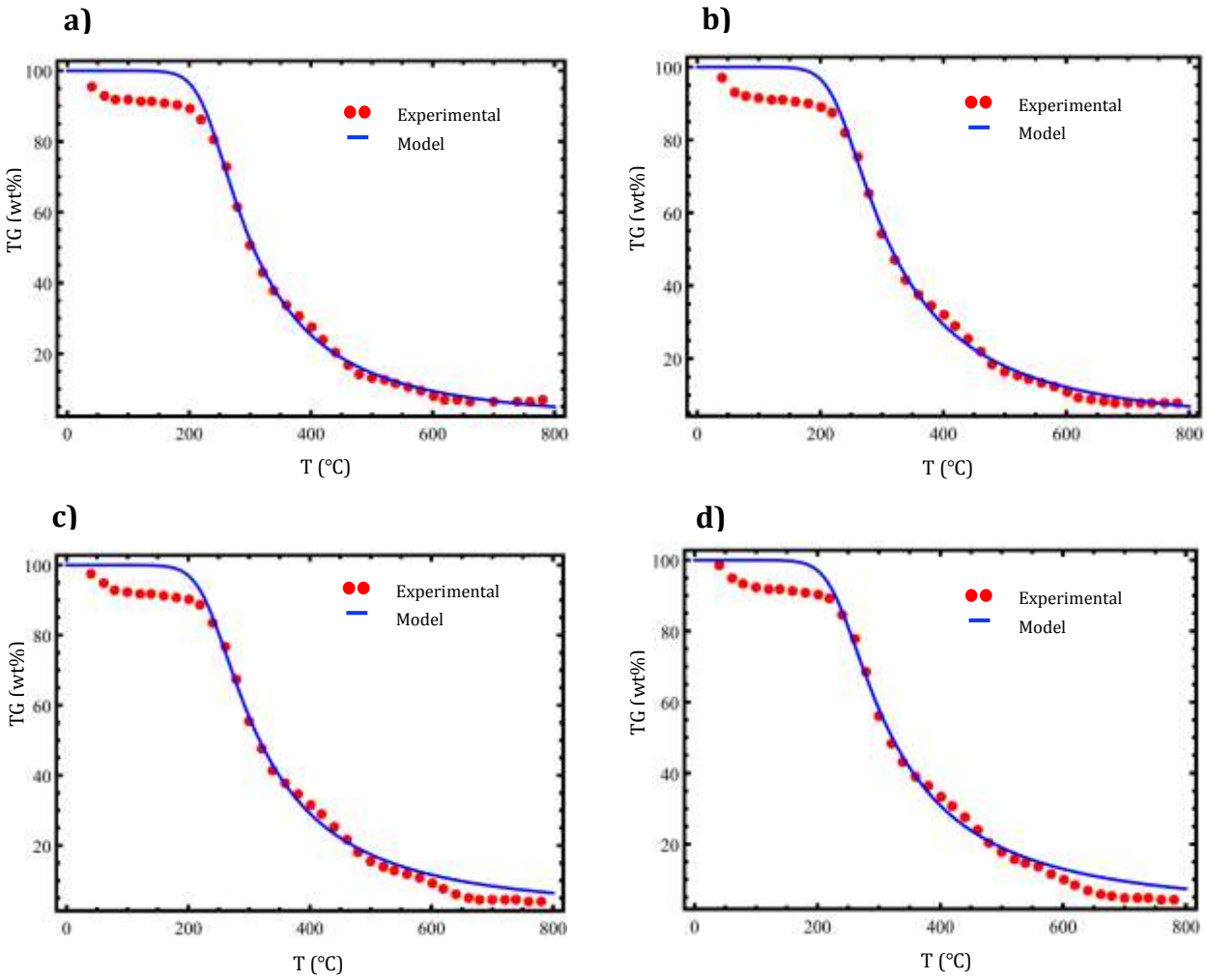


Figure 7-3 Experimental and Model TG Curves for *C. vulgaris* at a) β=5℃/min. b) β=10℃/min, c) β=15℃/min. d) β=20℃/min.

The estimated average reaction kinetic parameters described in Table 7-4, shows high value of reaction order (*n*), since the reaction orders were 6.58 and 5.76 for *N. oculata* and *C. vulgaris* respectively. A similar result of such high value of (*n*), when n^{th} order reaction model is used was reported by Kirtania & Bhattacharya [128] in theirs study of the pyrolysis kinetics of fresh water alga strain *Chlorococcum humicola*.

In addition, the approximate analytical solution developed by Coats-Redfern method gave identical TG curve to those evaluated by the numerical solution generated using NDSolve command in Mathematica 9 software, which is mean that the Coats-Redfern method is an accurate approximation, and it can be used safely.

Table 7-3 Estimated Reaction Kinetic Parameters Using the nth Order Model Fitting.

	N. oculata				*C. vulgaris*			
β	k_o, min^{-1}	n	E_a, kJ/mol	R^2	k_o, min^{-1}	n	E_a, kJ/mol	R^2
5	3.38 x 10^9	6.06	106.43	0.88	3.75 x 10^8	5.51	95.77	0.88
10	4.28 x 10^9	6.49	105.66	0.85	3.32 x 10^8	5.91	92.88	0.85
15	9.14 x 10^9	6.79	107.72	0.83	3.62 x 10^8	5.70	92.156	0.85
20	1.04 x 10^{10}	6.98	107.65	0.80	4.60 x 10^8	5.95	92.04	0.84

Table 7-4 Comparison between Average Reaction Kinetics Parameters Evaluated Using nth Order Model from Previous Studies and This Work.

Microalgae	β, ^{o}C/min	Atmosphere	E_a, kJ/mol	k_o, min^{-1}	n	References
Chlorococcum humicola	5, 10, 20 and 80	Nitrogen	189.99	2.44 x 10^{16}	7.25	[128]
N. oculata	5, 10, 15 and 20	Air	106.87	6.80 x 10^9	6.58	Present work
C. vulgaris	5, 10, 15 and 20	Air	92.36	3.82 x 10^8	5.76	Present work

7.5. Evaluation of the Iso-conversional Methods

Using iso-conversional models KAS and FWO the activation energy was determined using different heating rate experimental data, hence the evaluation of apparent activation energy was done without any need to define the reaction mechanism and how the reaction rate look like [129].

The plot $\ln\left[\frac{\beta}{T^2}\right]$ versus $1/T$ for α values of 0.2, 0.4, 0.6 and 0.8 for KAS method shows for *N. oculata* in Figure 7-4a. Figure 7-4b shows similar plots for the species of *C. vulgaris*. E_a and k_o calculated from the slope and intercept of all lines produced for four different α values and shows in Table 7-5 for comparison. The plot of $\ln[\beta]$ versus $1/T$ for α values of 0.2, 0.4, 0.6 and 0.8 for FWO method shows for *N. oculata* in Figure 7-5a and for *C. vulgaris* in Figure 7-5b. The E_a and k_o by the same methods described before, and the values listed in Table 7-5.

From Table 7-5 it can be noted that the average apparent activation energy, E_a for *N. oculata* is found 149.22 kJ/mol by KAS method and 151.78 kJ/mol by FWO method. For the case of *C. vulgaris* it is found 214.35 kJ/mol by KAS method and 213.44 kJ/mol by FWO method. Obviously, the values of activation energy for is *C. vulgaris* much greater than *N. oculata*, however this results is limited to biomass under study, since changing culturing condition and growth parameter may reflect on the produced biomass composition and therefore the thermal characteristics of biomass decomposition.

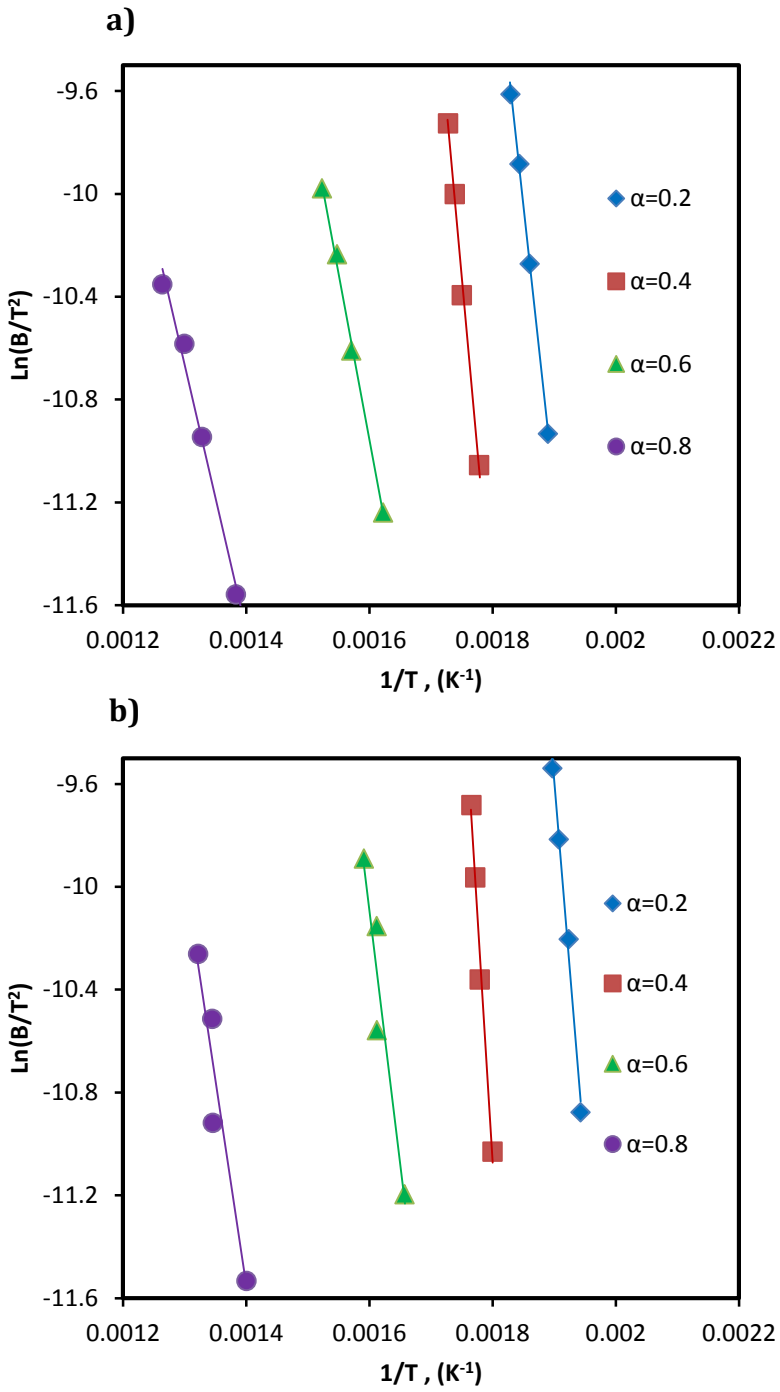


Figure 7-4 The Plot of ln[β/T^2] Versus 1/T for Different α Values for KAS Method for a) *N. oculata*, b) *C. vulgaris*.

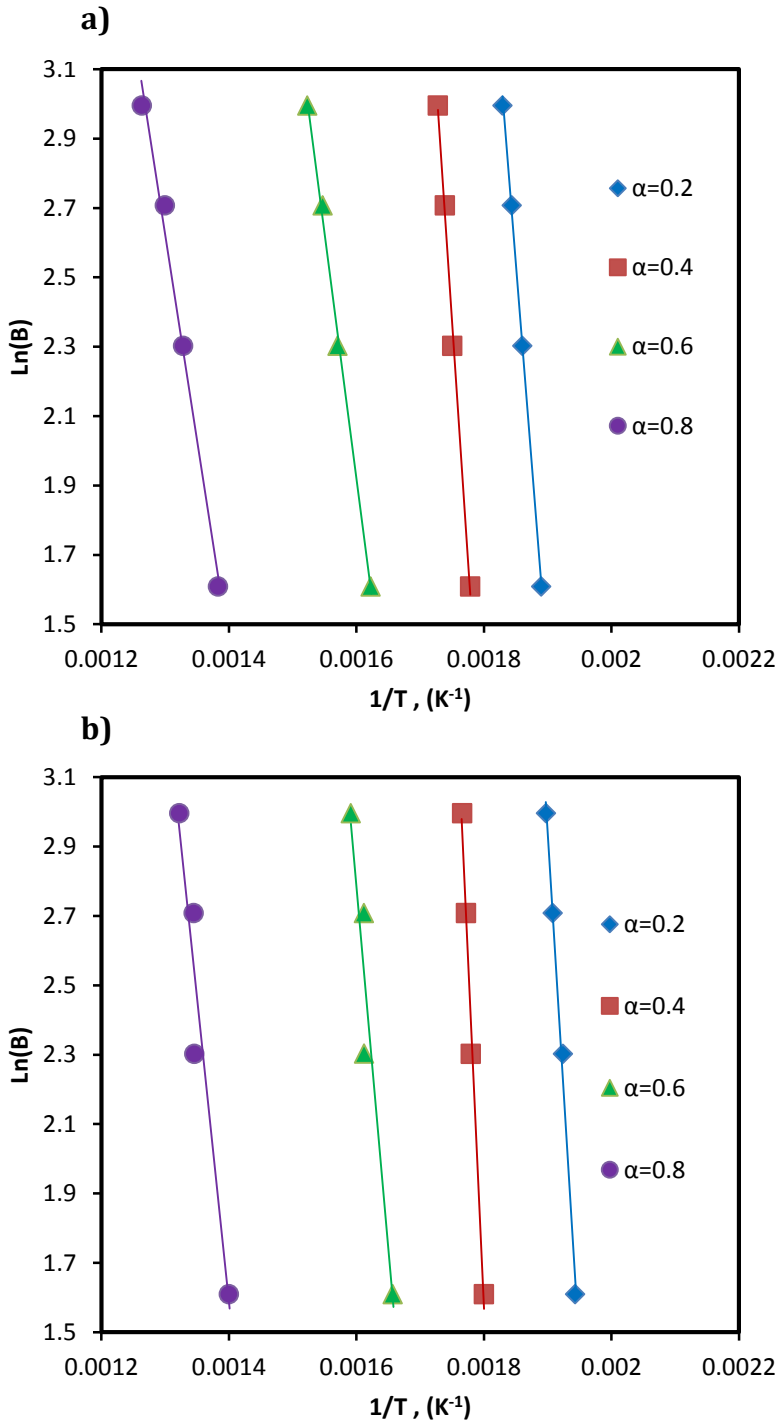


Figure 7-5 The Plot of ln[β] Versus 1/T for Different α Values for FWO Method for a) *N. oculata*, b) *C. vulgaris*.

From author's experience, that activation energy determined by KAS and FWO methods is very sensitive towards small changes in the experimental data than the model fitting techniques described before, however a considerable change are noticed in the average activating energy if experiments replicated. For all reviewed studies the difference between the average values of activation energy estimated by KAS and FWO are in significance for various microalgae biomass under different atmosphere with different heating rate used (Table 7-6).

Table 7-5 Apparent Activation Estimated Using KAS and FWO Methods

		KAS			FWO		
	α	Slope	E_a, kJ/mol	R^2	Slope	E_a, kJ/mol	R^2
	0.2	-21939	182.40	0.999	-23014.1	181.9503	0.999
	0.4	-26602.6	221.17	0.995	-27742.7	219.335	0.995
N. ocualta	0.6	-12919.6	107.41	0.996	-14189.9	112.1864	0.997
	0.8	-10334.7	85.92	0.987	-11845	93.6474	0.990
	Average		149.23			151.7798	
	0.2	-28873.2	240.052	0.992	-29914.6	236.51	0.993
	0.4	-38656.4	321.3896	0.986	-39777.6	314.48	0.987
C. vulgaris	0.6	-19607.9	163.0202	0.917	-20837.7	164.74	0.926
	0.8	-15990.9	132.9486	0.913	-17457.2	138.02	0.926
	Average		214.3526			213.45	

Table 7-6 Comparison between Reaction Kinetics Parameters Evaluated KAS and FWO from Previous Studies and Including This Work.

Microalgae	β, °C/min	Atmosphere	E_a, kJ/mol KAS	E_a, kJ/mol FWO	Reference
C. vulgaris	10, 20 and 40	O_2: N_2 (20:80)	134.53	134.03	[114]
C. vulgaris	10, 20 and 40	O_2: N_2 (50:50)	163.49	165.74	[114]
C. vulgaris	10, 20 and 40	O_2: N_2 (60:40)	211.24	209.92	[114]
C. vulgaris	10, 20 and 40	O_2: N_2 (80:20)	242.33	241.04	[114]
*Macrocystis pyrifera**	5, 10, 20, and 30	Ar	221.4	219.7	[113]
*Potamogeton crispus**	10, 30 and 50	N_2	143.2	145.3	[130]
*Sargassum thunbergii**	10, 30 and 50	N_2	185.6	185.4	[130]
Tetraselmis suecica	5, 10, and 15	O_2: N_2 (21:79)	70.09	75.81	[131]
C. vulgaris	10, 20 and 40	O_2: N_2 (20:80)	37.58	42.11	[120]
C. vulgaris	10, 20 and 40	O_2: CO_2 (20:80)	46.13	50.22	[120]
C. vulgaris	10, 20 and 40	O_2: CO_2 (50:50)	53.75	57.31	[120]
C. vulgaris	10, 20 and 40	O_2: CO_2 (80:20)	56.27	59.50	[120]
N. oculata	5, 10, 15 and 20	Air	149.23	151.78	Present work
C. vulgaris	5, 10, 15 and 20	Air	214.35	213.44	Present work

* Macroalgae

Although, the apparent activation energy is not constant and it varies during the conversion as clear in Figure 7-6, which means there is more than single reaction mechanism for the biomass thermal decomposition process [112]. Therefore, single reaction mechanism at each thermal decomposition stage can be used to evaluate the activation energy and other reaction parameters instead of single mechanism for the whole process [132].

To analyze the trend of activation energy versus reaction conversion we found that for all decomposition stage $\alpha = 0.2, 0.4, 0.6,$ and 0.8 the average estimated activation energy by KAS and FWO methods for *N. oculata* is lower than in that for *C. vulgaris*. A general trend is that at small α, activation energy increase with increasing the reaction conversion till reaching the maximum at almost a middle

conversion, then the value of activation energy decreases sharply until the end of combustion process as shown in Figure 7-6. Therefore, the facts that apparent activation energy change during the decomposition indicates the present of different reaction mechanisms throughout the thermal decomposition process, since these iso-conversional methods do no depend on a reaction mechanism for activation energy determination [112].

Multi stages kinetics modeling are normally being used for this purpose, since the thermal decomposition process is divided into devolatilization stage (which is further sub-classified) , oxidation stage, and last stage associated with the carbonate decomposition and metal volatilization [132]. Multi-zone pyrolysis analysis was also involved in study done by Wu et al. [116] for the thermal analysis and modeling of aquatic biomass decomposition including *D. tertiolecta* microalgae.

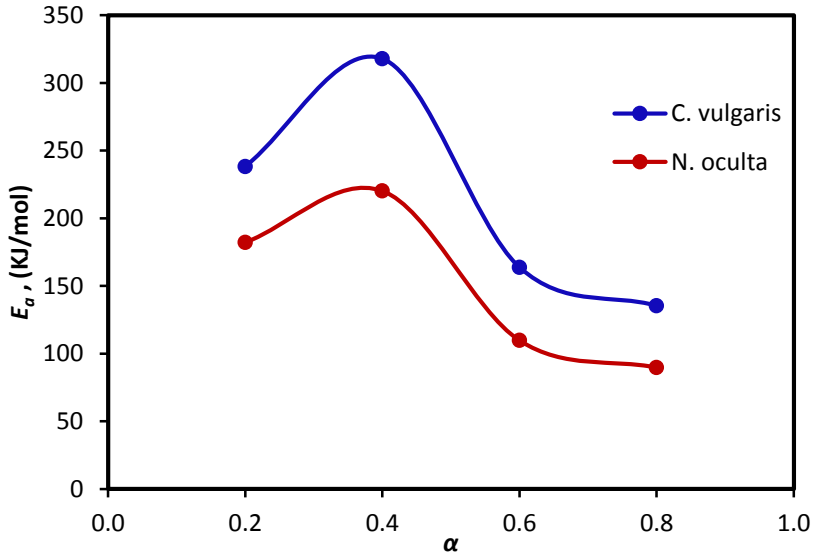


Figure 7-6 Average Activation Energy Estimated By KAS And FWO Methods at Different Conversion Stages for *N. oculata* and *C. vulgaris*.

7.6. Conclusion

- The results indicate that biomass of different microalgae strains exhibit different thermal behavior and characteristics.
- Growth parameters and medium composition can affect the biomass productivity and composition. This would have significant impact on the thermal decomposition trend of the biomass.
- The kinetic modeling of the oxidation reaction with direct model fitting method shows good prediction to the experimental data.
- The apparent activation energy estimated by KAS and FWO methods for *N. oculta* were 149.2 and 151.8 kJ/mol respectively, while for *C. vulgaris* were 214.4 and 213.4 kJ/mol respectively.

CHAPTER 8

LIPIDS EXCTRACTION

Biodiesel production from microalgae is considered as promising strategy, however production of such fuel consist of multiple processes which are cells cultivation, biomass harvesting, oil (lipids) extraction, and conversion of these lipids into fatty acid methyl esters (FAME) which is biodiesel [133]. Normally, lipids are extracted using either solvent extraction method or supercritical fluid extraction method, although the later approach is effective process but it is required very expensive equipment to be operated [134].

In facts, development of efficient extraction process is necessary in order to scale-up the lipid extraction and biodiesel production to industrial scale. An ideal extraction technology allow selective extraction of the desired lipids fraction without extraction of other contaminants, and with minimum energy for operation and avoidance of lipid degradation [135].

In this chapter, we investigated lipids extraction using n-hexane as organic solvent. Moreover, the effect of sonication on the quantity of lipids extracted was also been studied.

8.1. Lipids Content and Productivity

For the gravimetric determination method, the lipids content is calculated using the following equation (Eq. 8-1).

$$\text{Lipids content}(\%) = \frac{\text{Extracted lipids wieght}}{\text{Dry biomass wieght}}.100\% \qquad (8\text{-}1)$$

However, when wet biomass is used, it is very important to account for the moisture content. Hence, the dry biomass is then calculated using Eq. 8-2 as follows:

$$\text{Dry biomass weight} = \text{Biomass sample weight}(1 - \text{moisture fraction}) \qquad (8\text{-}2)$$

Furthermore, lipids productivity is a significant parameter defining the ability of microalgae to produce oil [44]. Lipids productivity can be calculated as the product between biomass productivity in g biomass/day and Lipids fraction in g lipids/g biomass (Eq. 3):

$$\text{Lipids productivity} = \text{Biomass productivity} \times \text{Lipids fraction} \qquad (8\text{-}3)$$

In solvent extraction methods, diffusion is main controlling mechanism for the extraction process, microalgae cell wall prohibit the solvent to diffuse inside the cells to extract the desired lipid, therefore cell disruption play important role in enhancing the lipid extraction efficiency [27].

Table 8-1 shows the extracted crude lipids without any pretreatment processing. As clear, the total lipids content and productivity are very low. Halim et al. [17]

describe the lipids extraction process using organic solvent method by the following steps: i) Diffusion of the solvent from the bulk through the stagnant liquid layer ii) Diffusion of the solvent through the cell wall iii) Solvent interact and dissolve lipids iv) Solvent and lipids dissolve through the cell wall v) Diffusion of solvent and lipids through the stagnant layer to the bulk solution. This mechanism is illustrated in Figure 8-1

Table 8-1 Lipids Content and Productivity without Cells Disruption.

Microalgae	Lipids content (%)	Biomass productivity (g/L/day)	Lipids productivity (mg/L/day)
N .oculata	6.85	0.077	5.28
C. vulgaris	3.12	0.081	2.12

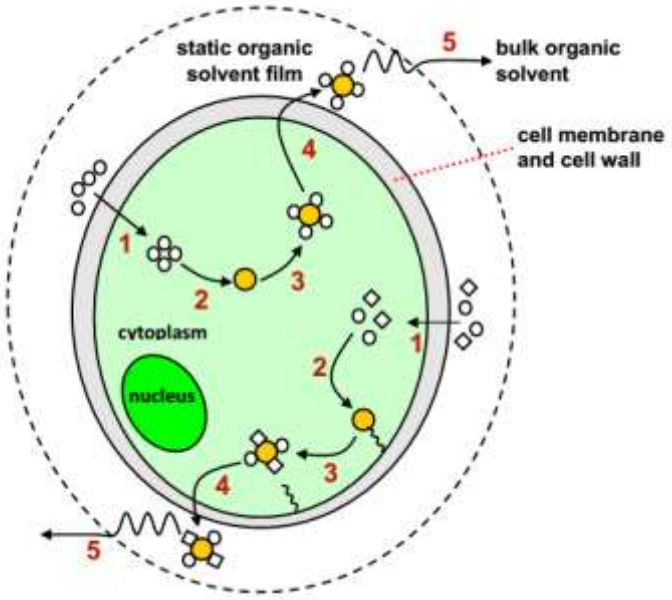


Figure 8-1 Mechanism of Lipids Extraction Using Solvent Method

8.2. Effects of Cells Disruption (Sonication)

Indeed, implementation of cells disruption techniques would enhance the extraction process, so several methods are available such as bead mills, sonication, autoclaving, osmotic shock, enzymatic digestion just to name a few [38]. However, lipids extraction assisted with ultrasonication gives higher extraction efficiency, minimizing extraction times and increase crude lipids yields, besides its acceptable costs [87].

However, when cell is disrupted, all these mass transfer resistances are eliminated. Therefore, in such cases higher lipids can be extracted. As consequence of sonication, lipids content has significantly increased. For *N. oculata* microalgae strain, the lipids content augmented from 6.85% to 23.38%, which represent around 3.4 times higher than lipids obtained without any pretreatment. On the other hand, lipids content of *C. vulgaris* went up from just 3.12 % to 10.17 %, again the enhancement in lipids yield is 3.25 approximately. Figure 8-2 describes the lipids content of both microalgae strains before and after sonication.

The same results was reported by de Souza Silva et al. [136] for the study of the effects of different extraction pretreatment processes on the lipids content of dry biomass. Since, the result out of the study showed that extracted lipids - from a mixed microalgae pond culture- upon using sonication, increase from 4.8wt% (no sonication) to 13.3 wt% (ultrasound applied).

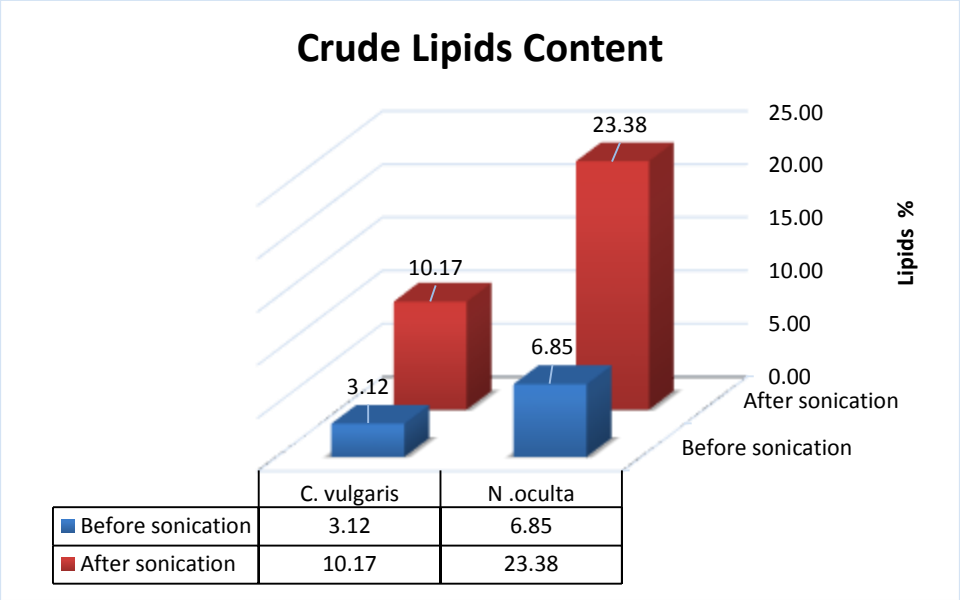


Figure 8-2 Extracted Crude Lipids Content

In addition, Keris-Sen et al.[137] investigated the effects of ultrasonication on the extracted crude lipids from biomass suspensions (wet biomass) of mixed microalgal cultivated in a BG11 (Blue-Green) medium. The extraction solvent was (1) *n*- hexane (2), which is a mixture of chloroform and methanol. The results showed that ultrasound augments the extracted lipids from biomass as: a) using *n*-hexane from 12 wt% (no sonication) to 17.6wt% (ultrasound applied), b) employing a chloroform and methanol from 13.6wt% (no sonication) to 26.8wt% (ultrasound applied) when a mixture is employed.

8.3. Conclusion

- Cell disruption methods can be used efficiently to improve the lipids extraction process.
- However, upon sonication the extracted crude lipids yield increase more than three times.
- The reported crude lipids content for microalgae strain *N. oculata* and *C. vulgaris* are 23.38% and 10.17% respectively.
- Therefore, *N. oculata* contain relatively high oil content, so it's appear as a possible candidate for biodiesel production.

CHAPTER 9

CONCLUSION AND RECOMMENDATIONS

9.1. Conclusion

Following are the conclusions of this study:

i. Culturing of microalgae species under phototrophic condition gives higher cells carbon content and rate of CO_2 capture than cultivation under mixotrophic mode.

ii. Based on the rate CO_2 bio-fixation, the integration of microalgae culturing unit within the wastewater plant should be on the tertiary stage.

iii. By culturing microalgae, phosphorus compounds are removed easier from wastewater than nitrogen compounds due to it's small concentration.

iv. Nitrogen compounds removal is a function of its initial concentration.

v. For both microalgae species phosphorus compounds were removed completely from wastewater.

vi. Biomass of different microalgae strains exhibit different thermal behavior and characteristics.

vii. Growth parameters and medium composition can affect the biomass productivity and composition.

viii. The kinetic modeling of the oxidation reaction with direct model fitting method shows good prediction to the experimental data.

ix. Cell disruption methods can be used efficiently to improve the lipids extraction process.

x. Using sonication, the extracted crude lipids yield increase more than three times.

xi. Therefore, *N. oculata* contain relatively high oil content, so it's appear as a possible candidate for biodiesel production.

9.2. Recommendations

The following recommendations are made based on the present study:

i. I would recommend to study further CO_2 biofixation for microalgae cultured in real municipal wastewater. Because in such case, biomass productivity and subsequently the rate of CO_2 capture would be lower side in comparison of what presented here.

ii. Investigating the effect of culture conditions such as CO_2 concentration, and concentration of other nutrients on the rate of CO2 capture.

iii. Developing a model to predict removal degree of nitrogen and phosphorus compounds as a function of their initial concentrations.

iv. Utilization of differed cells disruption methods.

v. Developing a novel catalyst to maximize the conversion of lipids to biodiesel.

APPENDICES

Appendix A: Basal Bold Medium (MBBM)

Table A-1: Basal Bold Medium (MBBM) [138]

Component	Conc. in stock solution	Volume for 1 Liter media	Conc. in final medium
$NaNO_3$	2.50 g per 100 ml	10 ml	2.94×10^{-3} M
$CaCl_2.2H_2O$	0.25 g per 100 ml	10 ml	1.70×10^{-4} M
$MgSO_4.7H_2O$	0.75 g per 100 ml	10 ml	3.04×10^{-4} M
K_2HPO_4	0.75 g per 100 ml	10 ml	4.31×10^{-4} M
KH_2PO_4	1.75 g per 100 ml	10 ml	1.29×10^{-3} M
NaCl	0.25 g per 100 ml	10 ml	4.28×10^{-4} M
EDTA KOH solution		1 ml	
Alkaline EDTA	5.0 g per 100 ml		1.71×10^{-4} M
KOH	3.1 g per 100 ml		5.53×10^{-4} M
Ferric solution		1 ml	
$FeSO_4.7H_2O$	4.98 per 100 ml		1.79×10^{-5} M
H_2SO_4	1 mL per 100 ml		
H_3BO_3	1.14 g per 100 ml	1 ml	1.85×10^{-4} M
Trace metal solution		1 ml	
ZnSO4.7H2O	8.82 g per 1 liter		3.07×10^{-5} M
MnCl2.4H2O	1.44 g per 1 liter		7.28×10^{-6} M
CuSO4.5H2O	1.57 g per 1 liter		6.29×10^{-6} M
CoCl2.6H2O	0.49 per 1 liter		-

Appendix B: Correction of TN-Ammonia measurement

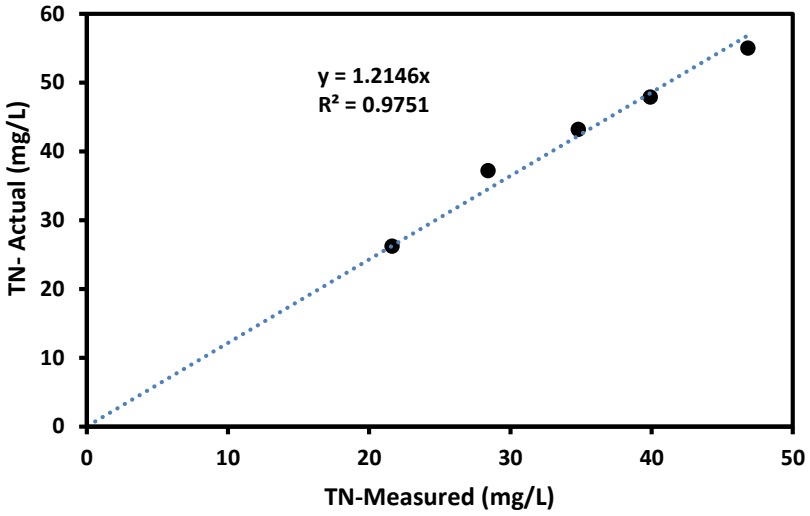


Figure B-1 TN-Ammonia correction

NOMENCLATURE

ASE	Accelerated solvent extraction
TAG	Triacylglyceride
FAME	Fatty acid methyl ester
TN	Total Nitrogen, (mg L^{-1})
TN-Ammonia	Total Nitrogen in Ammonia (NH_3) form, (mg L^{-1})
TP-Phosphate	Total Phosphorus in Phosphate (PO_4^{3-}) form, (mg L^{-1})
TP	Total Phosphorus, (mg L^{-1})
TGA	Thermogravimetric analysis
TOC	Total organic carbon, (mg L^{-1})
$TOC_{Biomass}$	Total organic carbon of microalgal biomass (g L^{-1})
$TOC_{Medium+Biomass}$	Total organic carbon of the culture medium containing the microalgae (g L^{-1})
$TOC_{Filtered\ Medium}$	Total organic carbon of the filtered medium (free of microalgae), (g L^{-1})
X	Biomass concentration (g L^{-1})
R_{CO_2}	Rate of CO_2 fixation (g L^{-1} day^{-1})

P	Biomass productivity (g L^{-1} day^{-1})
C_{CO_2}	Carbon content of microalgae biomass obtained from CO_2
M_{CO_2}	Molecular weight of carbon dioxide
M_C	Molecular weight of carbon
t_i	Cultivation time i (days)
t_o	Initial cultivation time (days)
W_f	Sample weight fraction remaining
α	Sample weight fraction burned (evolved)
T	Temperature (K)
T_{peak}	Maximum peak temperature (K)
R	Universal gas constant (kJ/mol K)
E_a	Apparent activation energy (kJ/mol)
k_o	Reaction rate constant1/min)
n	Reaction order
β	Heating (ramping) rate
R^2	Coefficient of determination

TG	Thermogravimetry
DTG	Differential thermogravimetry
KAS	Kissinger–Akahira-Sunose method
FWO	Flynn-Wall-Ozawa method

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2013 **Scholarship** for M.Sc. degree at King Fahd University of Petroleum & Minerals, Saudi Arabia.

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PUBLICATIONS:

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Razzak S.A., **Ali S.A.M.,** Hossain M.M., deLasa H. CO2 Capture and Biofuel Production from Microalgae Culture in Wastewater -A Review *(Submitted to Renewable & Sustainable Energy Reviews)*

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